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MOLD FUNGI IN THE ETIOLOGY OF RESPIRATORY ALLERGIC DISEASES

I. A SURVEY OF AIR-BORNE MOLDS

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A SERIES of cooperative studies was begun in 1939 between the department of botany and bacteriology, the University of Texas, and a group of physicians organized as The Association of Allergists for Mycological Investigation (Table I). One of these studies is in the nature of a survey of the air-borne molds in central and southwestern United States. The results over a two-year period of the survey are here reported.

The area included in this study consists of nine states in the mid-continental region of the United States extending from the Great Lakes to the Gulf Coast.* This region is especially well suited for comparison because of the contrasts in climate and topography. The participating stations were located in the following cities: El Paso,† Abilene, Fort Worth, Dallas, Waco, Temple, San Antonio, Houston, and Galveston in Texas; Shreveport, Louisiana; Little Rock, Arkansas;† Nashville, Tennessee; St. Louis and Kansas City in Missouri; Minneapolis, Minnesota; Milwaukee, Wisconsin; Evanston, Illinois; Toledo, Ohio.

METHOD

The plate method of aerobiologic analysis was used in this survey, and semimonthly exposures were made at each station. Whereas daily exposures may be made in a given locality to determine local environmental conditions, such frequency was thought neither advisable nor practicable for a more extensive survey, where trends of seasonal variation and geographic distribution over a wide area and for an extended

*This area at the present time is being extended to include new stations toward the Atlantic seaboard.

†Little Rock and El Paso were included in the survey only from April and June, 1940, respectively.

period are emphasized. Since all methods dependent upon gravity are generally regarded as qualitative rather than quantitative, and since emphasis in this survey was to be on the species making up the total mold count, this method was adopted;[1] at the same time, certain quantitative features are to be expected.

TABLE I

J. H. Black	Dallas, Texas
A. H. Braden	Houston, Texas
Maurice Barnes	Waco, Texas
W. H. Browning	Shreveport, Louisiana
Alan G. Cazort	Little Rock, Arkansas
L. O. Dutton	El Paso, Texas
Karl D. Figley	Toledo, Ohio
Sim Hulsey	Fort Worth, Texas
I. S. Kahn	San Antonio, Texas
Wm. L. Marr	Galveston, Texas
Tell Nelson	Evanston, Ill.
Edna Pennington	Nashville, Tennessee
Homer E. Prince	Houston, Texas
B. H. Reinarz	San Antonio, Texas
Erle Sellers	Abilene, Texas
Sam Schwartzberg	San Antonio, Texas
Boen Swinny	San Antonio, Texas
C. Malone Stroud	St. Louis, Missouri
T. L. Squier	Milwaukee, Wisconsin
A. R. Shearer	Mont Belvieu, Texas
Orval Withers	Kansas City, Missouri
A. Ford Wolf	Temple, Texas
Fred W. Wittich	Minneapolis, Minnesota

Isolated tall buildings were selected for exposure sites so as to avoid street level conditions. Standard 100 mm. Petri plates containing special wort agar were used.* Duplicate plates were taken to the exposure sites wrapped in sterile paper. To minimize local contamination from elevator penthouses and the like the open plates were held at arm's length over the side of the building toward the wind for two minutes. Plates received routine quantitative and qualitative study, and all new species from each station were recorded and preserved as stock cultures.

Monthly counts of total molds and dominant groups were expressed as an average of the duplicate plates on the two exposure dates.

RESULTS

All forms from a two-minute exposure should be considered dominant for that station under the prevailing weather conditions. A large number of different species of mold fungi may be considered dominant in the areas studied. Filamentous fungi belonging to the Phycomycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti were included in the count of total molds and studied as individual species. A number of these belong to the more common molds which are also found in the upper layers of soil, in house and street dust, and possibly as plant

*Formula from this laboratory, prepared by the Difco Company, Detroit, Mich.

pathogens. Yeasts, bacteria, and actinomycetes were not included although these were sometimes observed on the exposure plates selective for filamentous molds. All of the molds were studied and identified tentatively, and as many as possible have been verified as time and facilities permit.*

The count of total molds included all forms observed at a particular date. For convenience, the dominant forms were grouped into conventional categories. *Alternaria* was noted more often than any other one genus, and included *A. tenuis* Nees and *A. geophila* Daczewska, the former being the more commonly found. *Hormodendrum* was also of frequent occurrence throughout the area, being found mostly as *H. cladosporioides* Sacc., and less frequently as *H. hordei* Bruhne. Certain forms sometimes included in this genus have been designated as *Cladosporium*, *C. herbarum* Link being one of the species recognized. Certain others designated as *Monilia nigra* may also be included in the *Hormodendrum* group. *Helminthosporium* was found as *H. interseminatum* B. and Rav. and *H. halodes* Drechsler. Certain species which would seem to fall in the genus *Spondylocladium* and the related genus *Acrothecium* have been placed in *Curvularia* as *C. trifolii* Boedijn, *C. spicifera* Boedijn, *C. lunata*, and *C. brachyspora* Boedijn.

It is to be noted that the forms of more frequent occurrence encountered thus far in the survey are Dematiaceae species. *Stemphylium*, *Macrosporium*, and possibly *Tetracoccosporium* species have been found. One of the *Macrosporium* species isolated would seem to have a perfect stage related to *Phoma* or *Pleospora* and might be called *Camarosporium*. A species of *Stachybotrys*, and *Pullularia pullulans* Berkh., and *Nigrospora sphaerica* Mason were also found.

Of the Mucedinaceae species, the Aspergilli included *Aspergillus niger* Thom and Church, *A. flavus* Link, *A. nidulans* Eidam; the Penicillia, *P. carmino-violaceum* Dierckx, *P. chrysogenum* Thom, *P. digitatum* Sacc., *P. notatum* Westl., and *P. biforme* Thom; the Fusaria, *Fusarium scirpi* Lamb. et Fautr., *F. tricinctum* Sacc., and *F. sporotrichioides* Sherb. *Monilia (Neurospora) sitophila* Sacc., *Trichoderma lignorum* Harz., and species of *Paecilomyces*, *Gliocladium*, *Scopulariopsis*, *Botrytis*, and *Mycogone* were also observed.

The Mucorales were represented by species of *Rhizopus* and *Mucor*. A sterile Phycomycete probably belongs here. Species of the Ascomycetes producing perithecia as *Chaetomium* and others, and the Fungi Imperfecti producing pycnidia as *Phoma*, *Chaetomella*, and other "body"-forming species which do not fall into named categories, were also dominant forms. A number of isolations developed only sterile mycelium in culture, with no characteristic spore or other morphologic feature to aid in definite identification. Some of these suggested mushroom or other fleshy fungus mycelium or the vegetative mycelium of certain plant diseases.

In the compilation of the average counts in Figs. 1 to 5 a differentiation is made between southern and northern stations; southern stations include Shreveport, Louisiana, and those stations in Texas (except El Paso); northern stations include Kansas City, St. Louis, Nashville, Toledo, Evanston, Milwaukee, and Minneapolis. Fig. 1 shows the average counts for total molds in the southern and northern stations. Figs. 2 and 3 show the average counts at both southern and northern sta-

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tions for *Alternaria* and *Hormodendrum*, respectively. Fig. 4 shows the average counts for total molds, *Alternaria*, and *Hormodendrum* at the southern stations; Fig. 5 shows the same average counts for the northern stations.

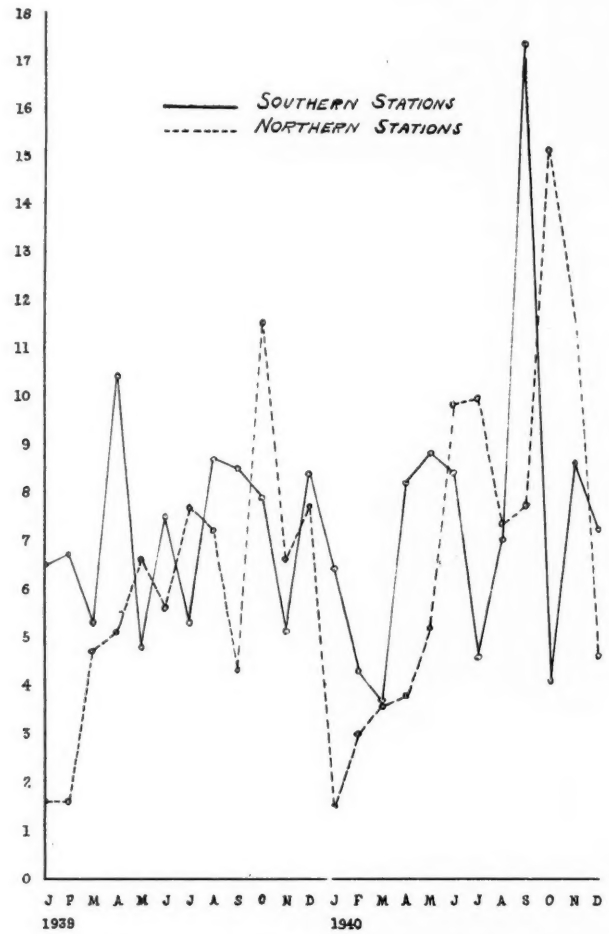

Fig. 1.—Average total mold counts at the southern and northern stations.

TOTAL MOLDS

Molds were recovered from every station during every month of the two-year period except St. Louis in February, Evanston in April, and Evanston and Milwaukee in May, 1940. In the January–June period of 1939 high counts persisted in the south with the highest occurring in April, whereas a contrast from winter low to higher counts in the spring was evident at the northern stations, the maximum monthly count equaling the southern count only in May (Fig. 1). During the last half of the year the counts in the north generally equaled or exceeded those in the south and rose to the highest peak of the year in October, while in the south only a slight increase over the spring period was recorded. Except for a decline in September at the northern stations and a similar one in November at the southern stations, the maxima for both regions occurred from August through December.

Highest counts* occurred as follows: at Abilene, 19 in April; at Nashville, 17 in May; at Kansas City, 26 in July; at Toledo, 29 in October; and at Abilene, 26 in December.

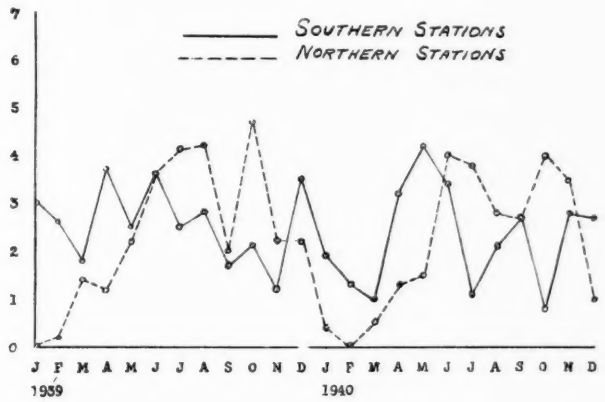

Fig. 2.—Average *Alternaria* counts at southern and northern stations.

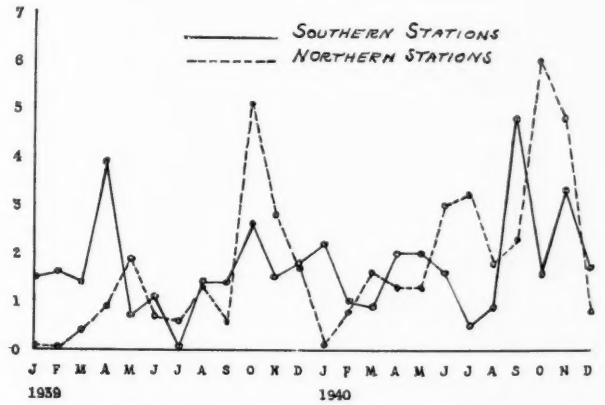

Fig. 3.—Average *Hormodendrum* counts at southern and northern stations.

With only slight variations similar trends were exhibited by both sections in 1940. In the south, while high counts occurred in May in the spring, the peak of the two-year period was reached in September. Mold occurrence in the north failed to reach the southern levels until June, after which, except for September and December, the counts exceeded those of the south. In the south the period of high counts apparently began in April or May, and in the north in June. Highest counts occurred as follows: at San Antonio, 29 in January; at Galveston, 24 in May; at Toledo, 25 and 41 in June and July, respectively; at Milwaukee, 27 in October.

The highest total count for the two-year period was observed at Toledo with 41 in July, 1940.

ALTERNARIA

Alternaria was encountered more frequently than any other one genus. It was recovered from every station and, with the exception of the north in the winter, was found at most of the stations every month.

*In discussing results only whole numbers are used.

In 1939 a high level was maintained in the south throughout most of the year, the counts increasing slightly to maxima in April, August, and December, decreasing during the fall (Fig. 2). In the north a gradual increase in counts began in February; these reached the level of the southern stations in June and exceeded those counts thereafter until December. The period of highest incidence in the

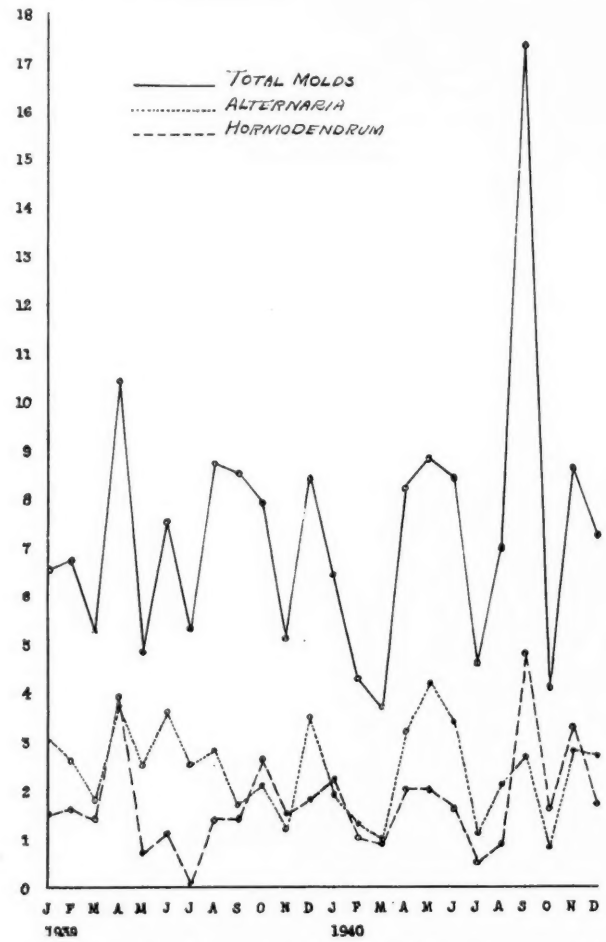

Fig. 4.—Average total mold, *Alternaria* and *Hormodendrum* counts at southern stations.

north extended roughly from June through October. During the January–June period a high count of 10 occurred in February at Temple, 10 in April at Houston, and 10 in June at Evanston. Highest counts in the July–December period were in July, October, and December at Kansas City, Toledo, and Abilene with 13, 13, and 11, respectively.

Similarly as in the corresponding period of 1939, *Alternaria* counts were higher at the southern stations during the winter and spring of 1940, increasing from March to a peak in May. In the north the trend of *Alternaria* occurrence exhibited during the preceding year was repeated in 1940, the counts rising gradually to a level in excess of that in the south in June and equaling or exceeding those in the south until December. The period of highest counts in the north, June through

November, was similar to that of 1939. High counts of 12, 14, and 18 respectively occurred at San Antonio in January, Galveston in May, and Toledo in July.

HORMODENDRUM

Hormodendrum ranked next to *Alternaria* in high counts and frequency of occurrence. It was recovered from every station during the two-year period and, except in the north during the winter months, was found more or less consistently at most of the stations.

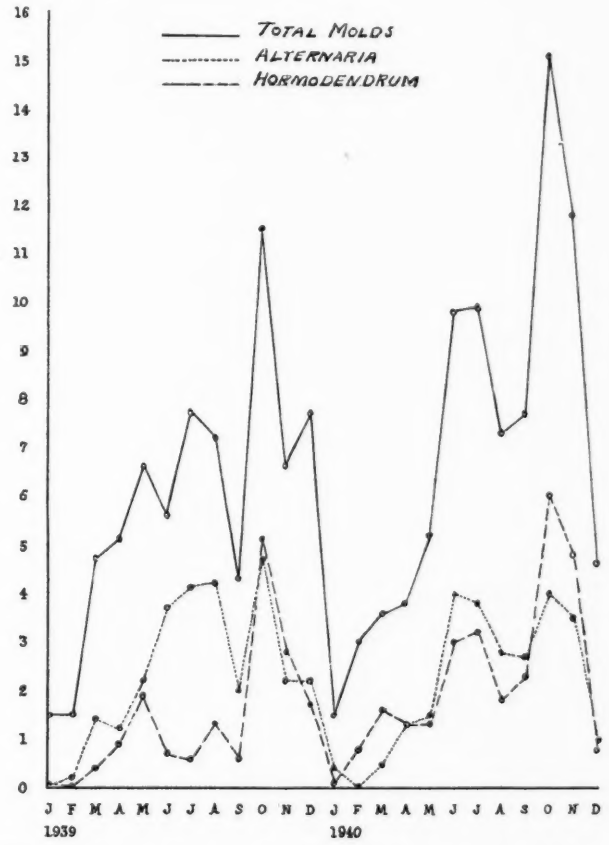

Fig. 5.—Average total mold, *Alternaria* and *Hormodendrum* counts at northern stations.

Maximum counts in 1939 for *Hormodendrum* in the south occurred during the winter, spring, and fall, the numbers diminishing in the late spring and summer. In the north this genus was practically nonexistent in January and February, but the counts increased in the spring in May, declined slightly in the summer, and increased again to a high in the fall in excess of the southern stations (Fig. 3). In both regions the periods of highest counts were in the spring and fall. The high count of 12 in 1939 occurred at Nashville in May and Toledo in October.

In the south in 1940, while moderately high levels were maintained in the winter and spring, a decline similar to 1939 was observed in the summer, the highest levels being reached in the fall. Also in the north the trend was similar to that of 1939,

rising to the southern levels in June and exceeding them throughout the summer and fall with the exception of September. During 1940 the maximum count of 13 occurred at San Antonio in January, 10 at Toledo in June, 18 at Toledo in July, 12 at Houston in September, and 12 at Toledo in October.

The highest count of *Hormodendrum* for the two-year period was found at Toledo in July, 1940.

Within limitations, the high totals observed at all of the stations and the decreased totals in the north during the winter months were reflections of the variations in the incidence of *Alternaria* and *Hormodendrum* (Figs. 4 and 5).

HELMINTHOSPORIUM

Helminthosporium was recovered from every station except Toledo and Evanston during the two-year period, and Little Rock during the portion of 1940 that this station was in the survey. Although its distribution was wide, the counts in contrast to *Alternaria* and *Hormodendrum* were generally low, and its occurrence was sporadic. During both years of the survey it was isolated most frequently in the south during the spring, summer, and fall, and in the north only in the summer. However, *Helminthosporium* was found repeatedly at San Antonio where it was observed consistently from June, 1939, to September, 1940, except in January and February. In 1939 the highest count of 4 occurred at Temple in September, and in 1940 the high of 4 was observed at Houston in September.

It would seem that both regional and seasonal factors are involved in the incidence of this genus, as it occurred most frequently in the south and in both sections the highest counts were found in the summer and fall.

SPONDYLOCLADIUM

The distribution and occurrence of *Spondylocladium* in general paralleled that of *Helminthosporium* throughout the two-year period. Except for Little Rock and El Paso it was isolated at every station.

In the January-June period of 1939 *Spondylocladium* occurrence was sporadic and confined chiefly to Texas, being found in the north only in June at Minneapolis. In the July-December period its incidence increased, and it was also observed in the north from July to October. In 1940 the trend was similar to that of 1939, except that its occurrence was somewhat more sporadic in the north during the summer.

The high counts of *Spondylocladium* were somewhat greater than those of *Helminthosporium*, the highest being 6 at Abilene in December, 1939. Although it was not encountered as frequently as *Alternaria* and *Hormodendrum*, *Spondylocladium* was encountered more frequently at Houston than at any other station during the two-year period.

Since the occurrence of *Spondylocladium* was sporadic during the winter in the south and since the maximum counts were confined to the summer and fall in both sections, a seasonal rather than a regional trend is suggested.

ASPERGILLUS

Aspergillus was isolated at all stations during the two-year period and was next to *Alternaria* and *Hormodendrum* in frequency of occurrence. Counts were relatively uniform and generally low, ranging from an average of 1 to 3, except for occasional moderately high counts at individual stations. Since it was observed only a little more frequently in the south and since it occurred as frequently in

the winter as in the summer, neither a regional nor a seasonal trend was apparent. However, it was recovered during five months or more per year at Dallas, Temple, St. Louis, Kansas City, and Houston.

The highest counts were 8 in 1939 at Dallas in September and 6 in 1940 at Temple in June.

PENICILLIUM

Penicillium, like *Aspergillus*, was encountered at every station and next to it in frequency of occurrence. Similarly, like *Aspergillus*, since counts were uniformly low throughout the two-year period and since it occurred only a little more frequently in the north than in the south, no seasonal or regional trend was suggested. It occurred with considerable regularity at St. Louis, Kansas City, and Toledo in both years, at Milwaukee in 1939, and at Minneapolis in 1940.

The high counts occurred at St. Louis in April, 1939, and at Toledo in May, 1940, with 5 and 4, respectively.

FUSARIUM

Fusarium was observed at all stations except Evanston and St. Louis and was similar to *Aspergillus* and *Penicillium* in frequency of occurrence.

Counts were generally low, and occurrence was sporadic throughout 1939. Except for the highs of 10 in August at Temple and 5 at Abilene in December, in no other case did the count exceed 3. In 1940, whereas counts did not tend generally to increase, *Fusarium* occurred more often at the various stations, particularly in the north from May until Fall. As in 1939 the counts never exceeded 3 except for the highs of 5 at Galveston in May, 9 at Houston in September, and 7 at Toledo in October.

The highest count of the two-year period was 10 which was observed at Temple in August, 1939. *Fusarium* was found more often at Houston than at any of the other stations, occurring there principally in the summer and fall.

Since *Fusarium* was encountered more frequently in the south than in the north with the exception of Minneapolis and since the counts were reduced in the south during the winter, a seasonal rather than a regional trend was suggested.

MUCORALES

The Mucorales, represented by *Rhizopus*, *Mucor*, and *Absidia*, were found at all stations except El Paso, Little Rock, Evanston, Nashville, Toledo and Galveston.

Counts were uniformly low and occurrence sporadic in both the north and the south throughout the two-year period. Except in 5 instances the counts never exceeded 1. Although the group occurred somewhat more frequently in the south, it was not found consistently in any region or at any station. The highest counts were noted at Fort Worth in November, 1939, and January, 1940, with 3 and 2, respectively. The counts were not so high, but occurrence was more frequent at Shreveport than at any other station.

The Mucorales were less pronounced in both counts and incidence than any of the other dominant groups, and neither a seasonal nor a regional trend was apparent.

"STERILE MYCELIUM"

"Sterile mycelium" was observed at every station during the two-year period but was not found at El Paso or Little Rock during the months these stations were studied.

Counts were generally low, rarely exceeding 2. The highest count of 5 was noted at Abilene in April, 1939; in 1940 the highest count was 3 at Galveston, in April.

There was no evidence of either seasonal or regional incidence.

"BODIES"

Phoma, Chaetomium, and other species producing "bodies" as pycnidia and perithecia, which do not readily fall into named categories, occurred as dominant forms and were recovered from all stations. Similar to "sterile mycelium," occurrence was sporadic and the counts were generally low. In only two instances did the counts exceed 2, these being found in 1939 at Kansas City in July, and Minneapolis in December with 3 and 4, respectively.

There was no indication of either a seasonal or a regional trend.

PULLULARIA

Pullularia, probably occurring mostly as *Pullularia pullulans* and appearing rarely in 1939, was not recovered as a dominant until the summer and fall of 1940. It exhibited no particular regularity but often occurred as a "shower" at the various stations, the comparatively high counts constituting a major fraction of the total.

The high counts of 8, 6, and 5 were observed respectively in 1940 at Evanston in February and at Minneapolis and Galveston in December.

DISCUSSION

Incidence.—The results emphasize the universal distribution of air-disseminated molds, regardless of geographic location, season, or climate. It is a common opinion that mold occurrence must be associated with particular conditions of climate, such as moisture and temperature. It is therefore difficult to realize the possibility of a mold problem, unless the weather is warm or damp. Molds are simply dismissed from the picture when the air is dry and the weather cold. This concept is readily understandable since it is not recognized that molds require very little water for the germination of spores or the stimulation of growth of mycelial fragments, as compared to seeds, for example, and the conditions required for their germination. In the two-year period, no extremes of temperature were reached at which some molds were not encountered. Actually, molds were recovered at one of the northern stations immediately after a heavy snow and following several days of near zero weather.

Habitat.—There is a prevalent idea that the Gulf Coast, a lake front, an open canal, or a bayou in a vicinity is conducive to mold growth and subsequent mold dissemination. To be sure, numbers may be increased by these factors, and the qualitative forms may be determined by, and be an expression of, particular sources in these environments. But occurrence is not dependent necessarily on these factors, for molds are encountered frequently in high areas and at considerable distances away from the sources usually thought most likely. It is a fact that microhabitats are often overlooked which might very well be responsible,

in part at least, for mold incidence in a given community. Mold spores will germinate and mycelial fragments will develop under conditions of surprisingly little moisture, in the absence of light, and in obscure places, such as storage basements, house foundations, dripping faucets, and leaf and other plant accumulations. High counts were found not only at Houston and Galveston, and at Toledo, where coastal and lake conditions might lead one to expect large numbers of molds, but were found also at the inland stations of San Antonio, Abilene, and Kansas City.

Individual Species.—The results indicate striking variations in the occurrence of the individual mold species. Certain species, such as *Aspergillus* and *Penicillium,* although numbers are relatively low, are of such common and consistent occurrence as to be designated cosmopolites. Others, as *Alternaria* and *Hormodendrum,* although recovered more frequently on the basis of yearly occurrence than any other genera, exhibit variations in incidence to such a degree as to be reflected in the regional and seasonal trends of the totals. Still others, as *Pullularia,* exhibiting neither regional nor seasonal trends, nor consistency in occurrence, suddenly appear and markedly affect the totals for a single count. This is in contrast to the concept that *Alternaria, Helminthosporium, Spondylocladium,* and *Hormodendrum* are the most commonly and consistently encountered forms.

Numbers.—Another fact which the results emphasize is that of the relatively high total numbers as fungus totals go. Although the plate method is not considered the best quantitative index to the mold content of the air, the fact that 41 mold spores are deposited on a 100 mm. agar plate in a two-minute exposure, or that 25, or 15, or even 1 fragment of mold mycelium is deposited there, would indicate the presence of relatively large numbers of molds in the air. It has been suggested[2] that the number of colonies developing on a standard Petri plate from a two-minute exposure may be transposed into terms of twenty-four-hour pollen slides, by multiplying the plate count by 21. Of course this considers only viable spores or mycelial fragments that will grow on artificial media and presumes a uniform distribution over the twenty-four hours.

SUMMARY

1. Molds are widely distributed throughout the central and southwestern United States.

2. Certain mold groups occur as dominant forms.

3. Total counts tend to be more uniform throughout the year in the south, reaching a peak in the fall.

4. The total counts in the north rise from a winter low to maxima in the summer and fall.

5. The high total counts in the north equal or exceed those in the south.

6. *Alternaria* and *Hormodendrum* are encountered more frequently and occur in higher numbers than any other molds.

7. With few exceptions the high total counts observed at all stations and the low total counts in the north during the winter are expressions of the variation of *Alternaria* and *Hormodendrum*. A seasonal rather than a regional trend in the case of these dominant species is indicated.

8. A seasonal trend is suggested in the occurrence of *Fusarium*.

9. Although *Aspergillus, Penicillium*, and certain other species are also encountered frequently, the numbers are low, and the occurrence is uniform throughout the year, neither seasonal nor regional trends being apparent.

10. *Pullularia* is striking in the fact that, when present, it occurs as a "shower" at different stations, making up a major fraction of the station total. Since this is observed during both winter and summer in the north as well as in the south, it would seem that the occurrence is local and not seasonal or regional.

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THE COMPARATIVE ATOPIC ACTIVITY OF ALTERNARIA SPORES AND MYCELIUM

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BOSTON, MASS.

NO STANDARD method has been devised for the production of mold extracts for testing and treating patients suffering from allergic respiratory disease, partly because it is not known whether the atopic excitant resides in the spores, the mycelium, or both. The purpose of this investigation is to determine what part of the mold is responsible for symptoms. *Alternaria* was chosen as the mold for study because it is widely accepted as a cause of respiratory allergy and because abundant reaginic sera and patients sensitive to *Alternaria* were available.

METHOD

It was desired to find a species of *Alternaria* which bore large quantities of spores on the surface of the mat and had a minimum of aerial mycelia. Such a mold occurred commonly on plates exposed to the outside air in Boston. Morphologically it appeared to be *A. tenuis*.

Spore Extracts.—By seeding spores into a flask of sterile physiologic solution of sodium chloride and then pouring a few cubic centimeters in each of many Petri dishes containing solid Sabouraud's medium, a uniform growth of mold was produced. After varying intervals of one to eight weeks the spores were harvested by brushing them loose from the underlying mycelium with a fine camel's-hair brush. They were then weighed. Microscopic examination revealed masses of single spores and almost complete absence of mycelium. It was found that within limits the longer the culture was permitted to grow at room temperature, the greater was the harvest of spores from a given area.

The spores were defatted twice with ether and then extracted for twenty-four to forty-eight hours in alkaline extracting fluid, followed by Seitz filtration. The protein nitrogen content of different batches of extract varied widely. In some instances there was no detectable protein nitrogen as evidenced by the failure of a precipitate to appear on the addition of phosphotungstic, sulphosalicylic, or trichloracetic acid, although total nitrogen content was comparable. The reason for this wide variation has not yet been determined. However, it afforded

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an opportunity to compare the atopic activity of extracts of equal total nitrogen content with and without protein nitrogen by direct testing of serial dilutions on *Alternaria*-sensitive patients.

Mycelial Extracts.—These were made from the same culture of *A. tenuis* as were the spore extracts. A few spores were seeded into flasks half full of liquid Sabouraud's medium, making sure that the spores were well below the surface of the fluid. Growth at room temperature was permitted for only thirty-six to forty-eight hours; then the small, fluffy balls of mycelium in the bottom of the flasks were collected on a Buchner filter and washed once with distilled water to remove as much of the medium as possible. Extraction of the mycelium in alkaline extracting fluid was carried out immediately for one-half hour before further growth or sporulation took place. The extract was then passed through a Seitz filter and standardized on a protein nitrogen basis. After extraction, the mycelium was collected on a piece of previously weighed filter paper and dried in an oven. The paper with mycelium was then weighed to determine the amount of mycelium from which the extract was made. Microscopic examination of samples of the mycelium before extraction showed only an occasional new spore. The bulk of material consisted of masses of mycelial threads.

The protein nitrogen content of the mycelial extracts varied widely as did that of the spore extracts. In general the mycelium yielded somewhat more protein nitrogen per unit weight than did the spores.

Spores vs. Mycelium: Direct Testing.—Twenty-five patients sensitive to *Alternaria* were tested intracutaneously with approximately 0.01 c.c. of spore and mycelial extracts of equal protein nitrogen content. In five experiments serial dilutions by multiples of five were tested.

Spores vs. Mycelium: Neutralization Experiments.—The sterile serums of six patients with negative Hinton tests and known to be sensitive to *Alternaria* were used either undiluted or in 1:10 dilution in the neutralization experiments. Two series of test tubes were set up, each containing an equal quantity of serum (or 1:10 serum) and antigen. The antigen in the first series consisted of spore extract in serial dilutions of multiples of two or five. The antigen in the second series was mycelial extract in corresponding dilutions and of the same protein nitrogen content as the spore extracts. The serum-antigen mixtures were incubated at 37° C. for one-half hour or left overnight in the icebox. The mixtures were then injected intracutaneously in 0.1 c.c. amounts on the upper arms of nonatopic recipients. The following day the serum-spore sites were tested intracutaneously with 0.02 c.c. of a spore extract containing 0.06 mg. of protein nitrogen per cubic centimeter. A few hours later the sites which failed to react, i.e., where neutralization was complete, were tested with 0.02 c.c. of mycelial extract containing 0.06 mg.

of protein nitrogen per cubic centimeter. Conversely the serum-mycelium sites were tested with mycelial extract, and later the neutralized sites were tested with spore extract.

RESULTS

Comparison of Spore Extracts With and Without Protein Nitrogen.—As indicated above, some batches of spore extracts failed to contain any protein nitrogen. The reason for this has not been found, but the age of the culture is not the determining factor. Because of this variation in protein nitrogen content of the extracts, an opportunity was afforded to compare the atopic strength of extracts with and without protein but of the same total nitrogen content by testing *Alternaria*-sensitive patients intracutaneously to serial dilutions. Ten subjects were tested with uniform results, as shown in Table I. Two facts are apparent. 1. The extract containing protein is at least twenty-five times more powerful as a skin-excitant than that without protein but with the same total nitrogen content. 2. The extract without chemically detectable protein contains a small quantity of atopically active substance.

TABLE I

INTRACUTANEOUS TESTS TO ALTERNARIA EXTRACTS WITH AND WITHOUT PROTEIN NITROGEN

NO.	PATIENT	TOTAL NITROGEN (MG./C.C.)					
		WITH 50% OF TOTAL NITROGEN AS PROTEIN NITROGEN			WITHOUT DETECTABLE PROTEIN NITROGEN		
		0.1	0.02	0.004	0.1	0.02	0.004
1	E. C.	+++	+++	++	+++	++	+
2	L. H.	++++	++++	+++	++	++	++
3	B. W.	++++	++++	++	++	+	0
4	W. H.	++++	+++	+++	++	++	+
5	D. C.	++	++	+	+	0	0
6	R. P.	++++	+++	++	+	+	0
7	M. R.	+++	++	++	++	+	±
8	J. D.	+++	+++	+++	++	+	±
9	J. S.	+++	+++	+++	++	±	±
10	R. L.	++++	++++	+++	+	0	0
Av. plus reaction		3.4	3.1	2.4	1.8	1.0	0.5

Spores vs. Mycelium: Direct Testing.—Spore and mycelial extracts, each containing 0.006 mg. of protein nitrogen per cubic centimeter, were compared by testing endermically twenty-five patients who were sensitive to *Alternaria*. Without exception, the spore extract elicited the larger response. The mycelial extract produced definite but smaller reactions in all but four instances, in which the reactions were no greater than in the controls.

In ten patients a mycelial extract ten times stronger (0.06 mg. of protein nitrogen per cubic centimeter) was compared with the spore extract containing 0.006 mg. of protein nitrogen per cubic centimeter.

In seven instances the weaker spore extract produced larger responses; in one instance the reactions were similar, and twice the mycelial extract produced the larger reaction.

Five subjects were tested to serial dilutions in multiples of five to the spore and mycelial extracts with essentially the same result; the spore extract was at least ten times stronger in atopic activity than the mycelial extract.

TABLE II

NEUTRALIZATION OF REAGINS TO SPORES AND MYCELIUM BY SPORE EXTRACT. FAILURE OF MYCELIAL EXTRACT TO NEUTRALIZE REAGINS TO SPORES

SERUM-SPORE MIXTURES			SERUM-MYCELIUM MIXTURES		
SITES PREPARED WITH 1:10 SERUM AND SPORE EXTRACT CONTAINING PROTEIN NITROGEN (MG./C.C.)	TESTED WITH SPORE EXTRACT*	TESTED WITH MYCELIAL EXTRACT*	SITES PREPARED WITH 1:10 SERUM AND MYCELIAL EXTRACT CONTAINING PROTEIN NITROGEN (MG./C.C.)	TESTED WITH MYCELIAL EXTRACT*	TESTED WITH SPORE EXTRACT*
0.06	0	0	0.06	±	+++
0.012	0	0	0.012	±	+++
0.0024	++	0	0.0024	±	+++
0.0005	+++		0.0005	++	
Control (normal skin)	0		Control (normal skin)	0	

*All testing extracts contained 0.06 mg. protein nitrogen per cubic centimeter.

Spores vs. Mycelium: Neutralization Experiments.—There were six experiments, all producing identically the same result. Table II represents one of them. Reagins to spores and mycelium were always present. The spore extract invariably neutralized both types of reagins, whereas mycelial extracts usually neutralized mycelial reagins but never affected the spore reagins.

SUMMARY

1. By direct testing of ten patients sensitive to *Alternaria* it was found that at least 95 per cent of the atopic excitant in the spores resides in the protein nitrogen.

2. After devising a method of making extracts purely from spores and from mycelia, it was determined by direct testing of twenty-five patients sensitive to *Alternaria* that the spores are at least ten times stronger in atopic excitant than the mycelium.

3. By in vitro neutralization experiments it was evident that reagins were present for both spores and mycelium. Whereas mycelial extracts neutralized only mycelial reagins, spore extracts invariably neutralized both spore and mycelial reagins.

4. Therefore, the atopic excitant in *Alternaria* resides in the protein portion of the spore, and extracts for testing and treating atopic patients should be made from spores instead of the whole mat, which includes a large amount of incomplete antigen from mycelium.

AIR-BORNE FUNGUS SPORES

A Five-Year Survey of Daily Mold Spore Content of Chicago Air

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Chicago, Ill.

THE realization of the importance of atmospheric mold spores as etiologic agents in seasonal allergy convinced us several years ago of the necessity for conducting a survey of the mold spore content of outdoor air. After we reported the results of a year of air observations,[1] it was deemed advisable to continue the studies over a period of several years. It was felt that the data gathered by a prolonged study would aid materially in correlating clinical symptoms with various types of fungi and in ascertaining qualitative and quantitative fluctuations within the season and from year to year. These findings finally would point the way to the selection of the proper antigens for diagnosis and treatment and would indicate the degree of protection desired for each type of fungus and the time of the year when the maximum dose should be reached.

A previous report of daily atmospheric mold spore counts by the culture plate method covered the thirteen-month period from November, 1934, to December, 1935.[1] Other communications on mold counting have been published by Prince and Morrow,[2] Pratt,[3] Schonwald,[4] Pennington,[5] and Waldbott and his associates.[6] These surveys have covered periods from six months to one year; sometimes the periods were discontinuous, and in some instances the results were based upon air sampling carried on as infrequently as once every two weeks. It is felt, therefore, that the presentation of a report based upon daily observations for a continuous period of five years (from 1935 to 1939 inclusive) might give a more complete picture of air contamination.

METHOD

Petri plates, 3.5 inches in diameter, containing potato-dextrose agar, were exposed horizontally several feet outside a window on the sixteenth floor of the Medical School Building. Two plates were exposed daily, consecutively for a period of fifteen minutes each. After incubation at room temperature for three or more days, depending upon the growth, the plates were examined and the colonies identified and counted. Identification of the colonies was made as to genus, and, as far as possible, the species also was recognized. For this presentation, however, the

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classification of results in terms of genera only will be considered; otherwise, the tabulation would become too complex and cumbersome. Furthermore, as a result of experiences with passive transfer skin tests and observations of symptoms, we have come to a fairly definite impression that species specificity is not a striking finding in mold allergy. In some instances, cross-desensitization has occurred even between different genera.

It is not practical to present in this paper anything more than the findings obtained from culture plate examinations. Slide exposures were used during the period discussed, but the results will not be presented here. In general, it may be said that each method, the plate and the slide, has its advantages and disadvantages and that neither is perfect in itself or even combined with the other. The slide method gives probably a more accurate count because of the twenty-four-hour period of its contact with the air; it shows some spores which would not grow on plates[7] because of lack of viability, and it presents for direct study spores, such as rusts and smuts, which do not produce colonies on artificial media. Slides can be stored for leisurely and repeated study. The chief disadvantage of the slide method is that the large varieties of small and indifferent appearing spores cannot be distinguished; the colonies can be recognized readily on the culture plates. Ordinarily, each method should be used to supplement the other. Even then we feel that in the atmosphere there are undoubtedly a number of fungus spores which will neither grow on our culture plates nor, because of their transparency, size, or lack of markings, be recognized on the slide. We feel that this is the probable explanation for the failure to obtain skin tests and clinical tests with the numerous available fungi or pollen in some patients whose seasonal clinical history suggests fungi as a cause of symptoms.

The period of exposure used is obviously a practical compromise. Continuous exposure of plates at fifteen-minute intervals throughout the twenty-four hours would furnish the maximum information concerning the fluctuations in the spore content of the air. Such a procedure, however, would not be practical over a prolonged period. However, by following such a technique for several days, with associated observations of temperature, wind direction and velocity, barometric pressure, and humidity, valuable data can be obtained, as suggested by the work of Cadrecha and Quintero.[8] We feel that at least daily exposure of plates is necessary in order to determine fluctuations within the season and from season to season. We admit that even then the method is not perfect, but at least it gives much more reliable information than can be obtained from exposures made infrequently and as far apart as two weeks.

RESULTS

For the purpose of recording the figures and presenting the results, several charts are shown. These comprise yearly curves for each of the four more commonly encountered genera, namely *Alternaria, Hormodendrum, Penicillium,* and *Aspergillus.* The daily number of colonies of each variety for the week and the average daily number of colonies for the week were computed and a curve constructed. The total number of mold colonies for each genus during the month is also represented on the chart. Curves on other charts show the incidence of the miscellaneous mold colonies and the total molds. Some of the miscellaneous molds (*Monilia, Botrytis, Mucor, Rhizopus, Phoma, Trichoderma, Cephalosporium,* and *Helminthosporium*) are considered separately, and their incidence on the basis of the total number of colonies per month is shown.

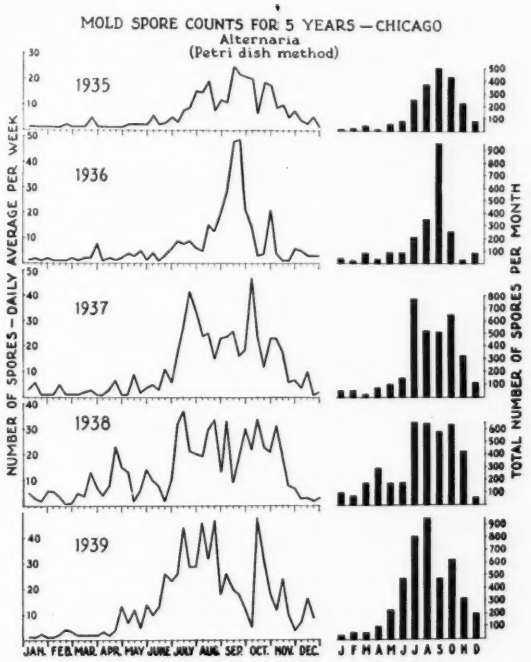
MOLD SPORE COUNTS FOR 5 YEARS — CHICAGO
Alternaria
(Petri dish method)
1935
1936
1937
1938
1939
NUMBER OF SPORES - DAILY AVERAGE PER WEEK
TOTAL NUMBER OF SPORES PER MONTH
JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEP. OCT. NOV. DEC.
J F M A M J J A S O N D

Fig. 1.

Alternaria (Fig. 1).—Although *Alternaria* spores were found in the air throughout the year, a decided seasonal tendency was apparent. In general, for the years studied, the greatest concentration of mold spores occurred during the months of June, July, August, September, October, and November. Eighty per cent of the total annual crop of *Alternaria* spores were found in this six-month period. But in some years, large numbers of spores occurred in other months. For example, in 1938, the number of spores in the air in March, April, or May was as great as

or greater than in June. In 1936, the period of maximum atmospheric contamination was of short duration, during the months of July, August, September, and October. The total number of *Alternaria* spores in September of that year was over 900; this was the largest number of these spores encountered during any month of the five-year period. During November of that year, the number of spores in the air was very small. Besides the variations in seasons from year to year, there also occurred fluctuations from week to week during any particular season. These fluctuations were due to the effects of variations in wind direction and velocity, temperature, sunlight, and moisture.

We wish to emphasize again that for *Alternaria* as well as for the other fungi we have daily counts and daily data which show even wider fluctuations, a sample of which can be seen on a curve in a paper published by us several years ago.[1] The short and long term mold count variations and the effect of various weather factors on them will be presented in another communication.

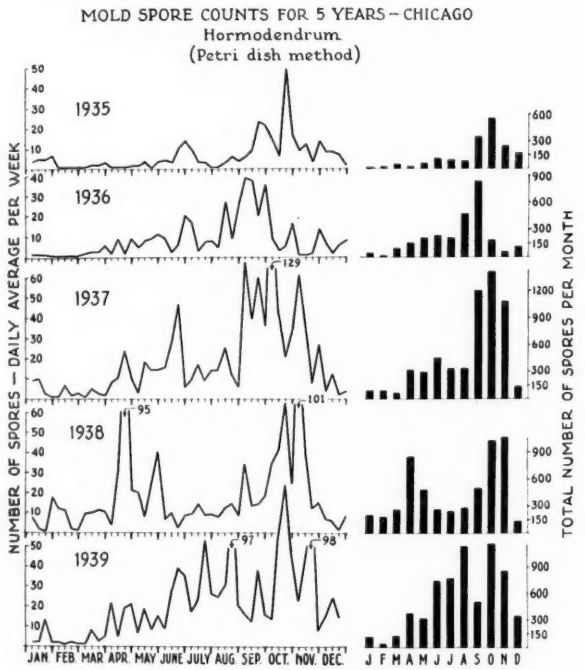
MOLD SPORE COUNTS FOR 5 YEARS – CHICAGO
Hormodendrum
(Petri dish method)
1935
1936
1937
1938
1939
NUMBER OF SPORES – DAILY AVERAGE PER WEEK
TOTAL NUMBER OF SPORES PER MONTH
JAN FEB MAR APR MAY JUNE JULY AUG SEP OCT NOV DEC
J F M A M J J A S O N D

Fig. 2.

Hormodendrum (Fig. 2).—The occurrence of the spores of *Hormodendrum* followed a seasonal distribution similar to that of the spores of *Alternaria*. However, the fluctuations in the time and duration of the seasonal increments from year to year were more marked than in the case of *Alternaria*. In April, 1938, the total number of *Hormodendrum* colonies found on the plates was exceeded only by the num-

bers encountered during the months of October and November. In general, the *Hormodendrum* season started earlier (in April) and was longer than the *Alternaria* season.

Aspergillus (Fig. 3).—Examination of Fig. 3 shows the lessened incidence of these spores as compared with the two previous ones. The lack of a seasonal tendency is noteworthy. The distribution through the year was fairly uniform, with occasional high counts that occurred at any season of the year. These exacerbations were probably correlated with various weather factors.

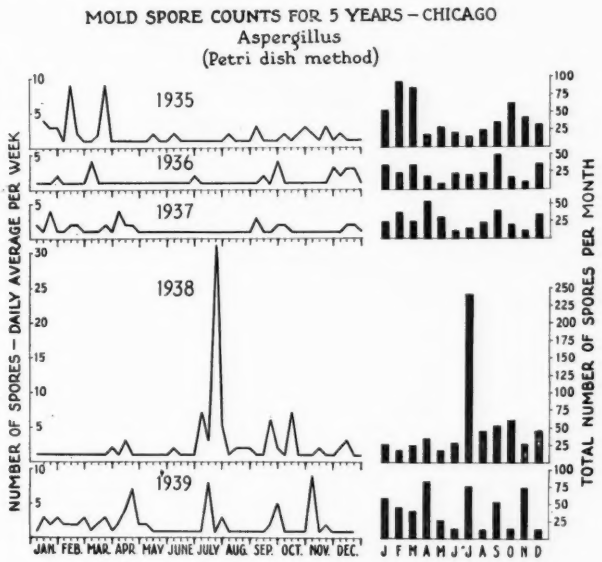
MOLD SPORE COUNTS FOR 5 YEARS – CHICAGO
Aspergillus
(Petri dish method)
1935
1936
1937
1938
1939
NUMBER OF SPORES – DAILY AVERAGE PER WEEK
TOTAL NUMBER OF SPORES PER MONTH
JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEP. OCT. NOV. DEC.
J F M A M J J A S O N D

Fig. 3.

Penicillium (Fig. 4).—The *Penicillium* curve exhibits characteristics very much like those of *Aspergillus*. The incidence of penicillium is greater and constitutes about 11 per cent of the total mold spore contamination. Its occurrence also is characterized by a lack of tendency to seasonal fluctuations. About 51 per cent of the total number of *Penicillium* colonies were counted during the six-month period from June to November. This may be compared with the figure of 80 per cent given previously for *Alternaria* for the same period.

Miscellaneous Molds (Figs. 5 and 6).—Some of the more commonly occurring miscellaneous spores, such as *Monilia, Botrytis, Mucor, Rhizopus, Phoma, Trichoderma, Cephalosporium,* and *Helminthosporium* are shown with reference to the total number of colonies for each month for the five years. Most of these molds exhibited no particular type of seasonal distribution. Others, as, for example, *Helminthosporium* and *Botrytis,* showed high counts during the summer and fall months. Marked variations in the number of these miscellaneous molds occurred

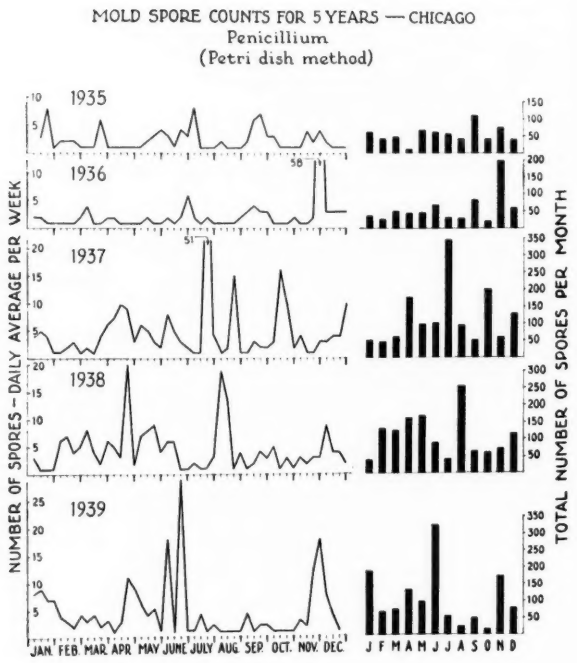
MOLD SPORE COUNTS FOR 5 YEARS — CHICAGO
Penicillium
(Petri dish method)
1935
1936
1937
1938
1939
NUMBER OF SPORES – DAILY AVERAGE PER WEEK
TOTAL NUMBER OF SPORES PER MONTH
JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEP. OCT. NOV. DEC.
J F M A M J J A S O N D

Fig. 4.

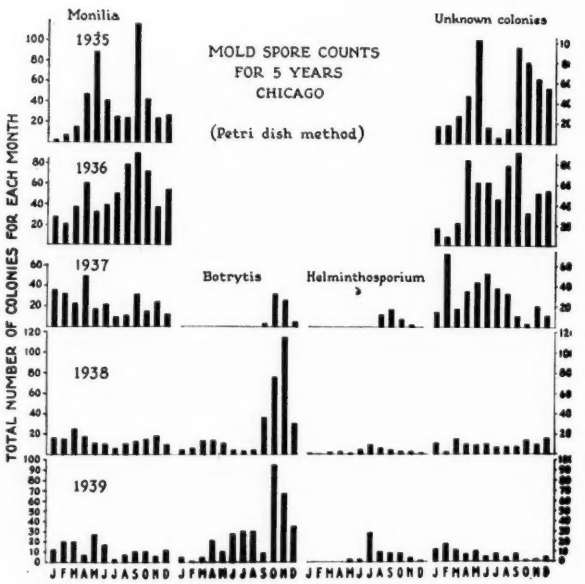
Monilia
Unknown colonies
MOLD SPORE COUNTS
FOR 5 YEARS
CHICAGO
(Petri dish method)
1935
1936
1937
1938
1939
Botrytis
Helminthosporium
TOTAL NUMBER OF COLONIES FOR EACH MONTH
J F M A M J J A S O N D

Fig. 5.

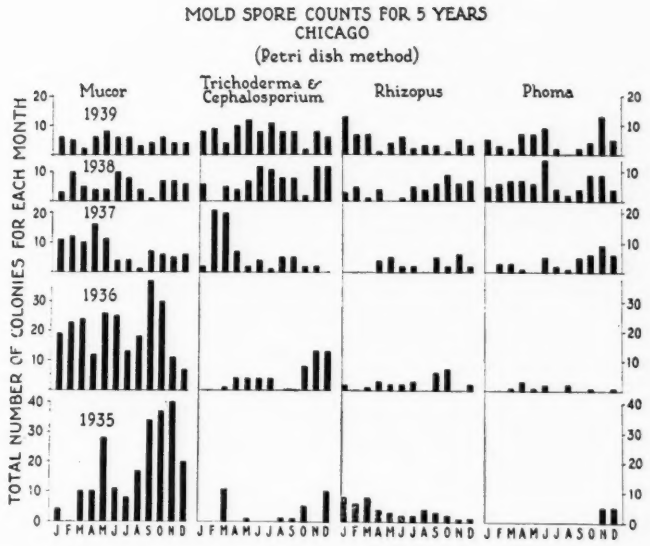
MOLD SPORE COUNTS FOR 5 YEARS
CHICAGO
(Petri dish method)
Mucor
Trichoderma & Cephalosporium
Rhizopus
Phoma
1939
1938
1937
1936
1935
TOTAL NUMBER OF COLONIES FOR EACH MONTH
J F M A M J J A S O N D

Fig. 6.

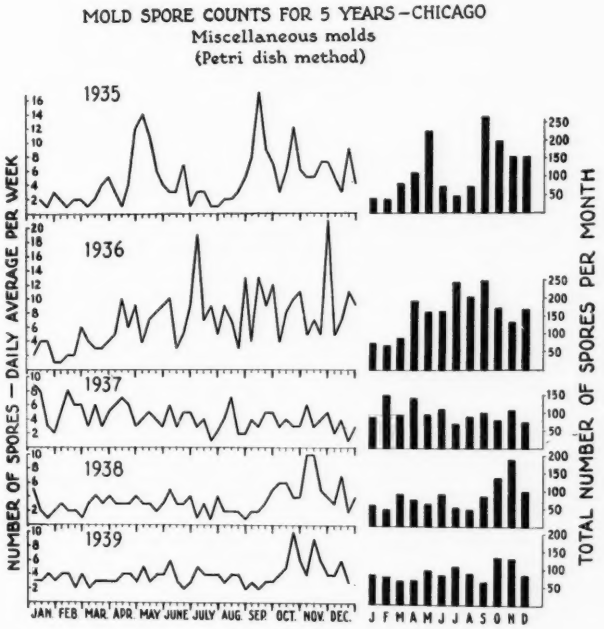
MOLD SPORE COUNTS FOR 5 YEARS—CHICAGO
Miscellaneous molds
(Petri dish method)
1935
1936
1937
1938
1939
NUMBER OF SPORES — DAILY AVERAGE PER WEEK
TOTAL NUMBER OF SPORES PER MONTH
JAN FEB MAR APR MAY JUNE JULY AUG SEP OCT NOV DEC
J F M A M J J A S O N D

Fig. 7.

from year to year. This was especially true for *Monilia* and *Mucor*. Fig. 7 is a representation of the miscellaneous molds considered as a group, with curves showing the daily average number of spores per week and the total number of spores per month. The lack of a tendency to seasonal occurrence is quite evident.

Total Mold Colonies (Fig. 8).—These curves show the seasonal fluctuations that have been demonstrated to be characteristic of the spores of *Alternaria* and *Hormodendrum*. The reason for this is understood when it is considered that these two molds constituted 72 per cent of the total mold spore crop. Since the other molds were in the air more or less constantly throughout the year, their effect on these curves was to make them more smooth, and with less pronounced peaks and dips.

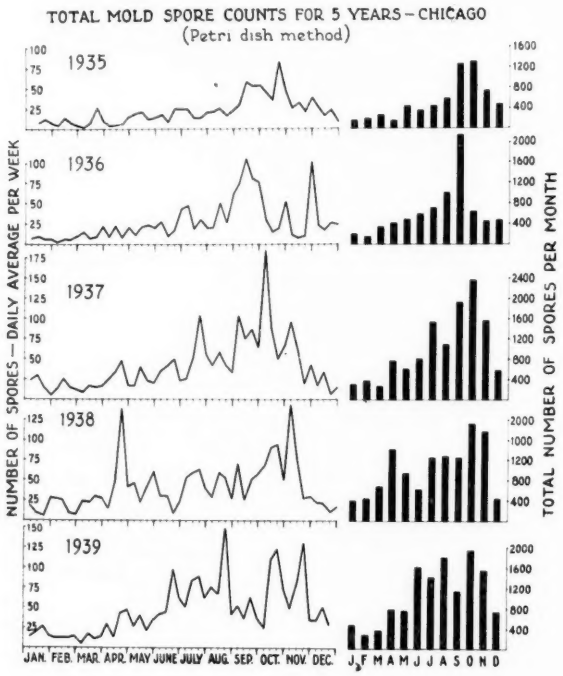
TOTAL MOLD SPORE COUNTS FOR 5 YEARS – CHICAGO
(Petri dish method)
1935
1936
1937
1938
1939
NUMBER OF SPORES – DAILY AVERAGE PER WEEK
TOTAL NUMBER OF SPORES PER MONTH
JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEP. OCT. NOV. DEC.
J F M A M J J A S O N D

Fig. 8.

The total number of mold colonies found on the plates for each year was computed, and tables were made showing the number for each type of mold for each year (Table I). Although the total number of colonies increased from year to year, the percentage of each genus comprising a part of the whole remained quite constant for each year. For example, *Alternaria* made up 32 per cent of the total mold count in 1935, 29 per cent in 1936, 28 per cent in 1937, 32 per cent in 1938, and 32 per cent in 1939. The percentages of *Penicillium* and *Aspergillus* similarly were quite constant. *Hormodendrum*, however, varied somewhat from year to year, as it comprised 28 per cent of the total number in 1935, 34 per

cent in 1936, 48 per cent in 1937, 44 per cent in 1938, and 47 per cent in 1939. The average mold count per year for each genus for the five-year period was computed (Table II). The average total number of mold colonies per year was 10,460. Of this average figure, the aver-

TABLE I

MOLD	TOTAL NO. OF MOLD COLONIES				
	1935	1936	1937	1938	1939
Alternaria	2,073	2,219	3,376	3,982	4,331
Hormodendrum	1,771	2,609	5,788	5446	6,449
Penicillium	637	627	1,399	1,317	1,295
Aspergillus	502	273	322	610	511
Miscellaneous	1,462	1,929	1,197	1,047	1,097
Total	6,444	7,654	12,080	12,427	13,683

TABLE II

MOLD	AV. NO. OF COLONIES PER YEAR	%
Alternaria	3,198	30
Hormodendrum	4,415	42
Penicillum	1,056	11
Aspergillus	444	4
Miscellaneous	1,347	13
Total	10,460	

TABLE III

INCIDENCE OF MISCELLANEOUS MOLDS

MOLD	AV. NO. PER YEAR	%	% TOTAL
Unknown	343	25	3.2
Monilia	332	25	3.2
Botrytis	144	11	1.4
Mucor	137	10	1.3
Actinomyces	106	8	1.0
Rhizopus	41	3	0.4
Phoma	39	3	0.4
Trichoderma	35	2.6	0.3
Chaetomium	33	2.5	0.3
Cephalosporium	32	2.4	0.31
Helminthosporium	29	2.2	0.29
Fusarium	24	1.8	0.23
Cephalothecium roseum	20	1.5	0.2
Spicaria	19	1.5	0.2

age *Alternaria* count per year was 3,198, or 30 per cent of the total. *Hormodendrum* showed an average number of 4,415 colonies per year, or 42 per cent of the total. The average number of *Penicillium* colonies was 1,056 per year, or 11 per cent of the total, while *Aspergillus* with an average number of 444 colonies per year accounted for 4 per cent of the whole crop. The miscellaneous and unknown colonies made up the rest, 1,347 colonies, or 13 per cent. Table III enumerates the miscellaneous molds, the average number of colonies of each per year, and their percentage distribution.

Curves were drawn showing the occurrence of the four molds most frequently found through the year in Chicago (Fig. 9). These curves are based on the average monthly mold count for the five-year period. The seasonal nature of *Alternaria* and *Hormodendrum* is shown. The average *Hormodendrum* season started earlier (in April) and continued longer (through December) than the average *Alternaria* season. As pointed out before, the spores of *Aspergillus* and *Penicillium* showed no tendency to seasonal fluctuation and comprised a small amount of the total atmospheric mold contamination.

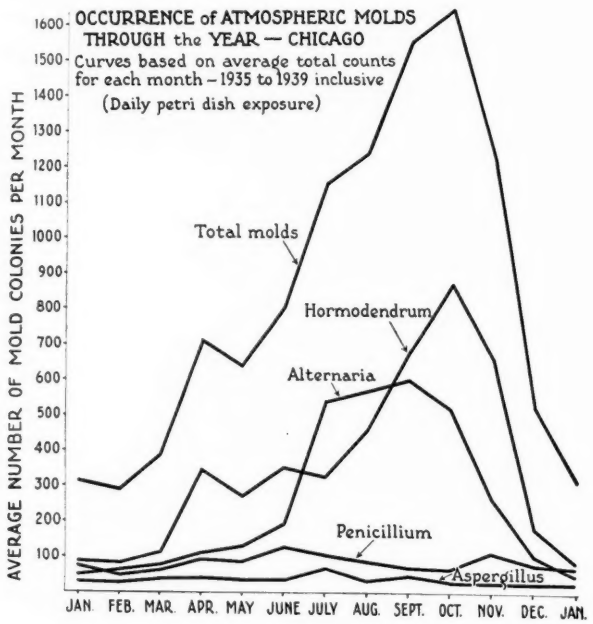

Fig. 9.

SUMMARY AND CONCLUSIONS

1. A daily study of the mold content of the air in the Chicago area for a continuous period of five years is presented.

2. *Alternaria* and *Hormodendrum* constituted 72 per cent of all the spores that were grown on artificial media.

3. Some fungi show no particular seasonal tendency, but those fungi that have a high incidence display marked seasonal fluctuations.

4. The seasons vary for the various fungi and also vary from year to year in length and in time of onset and termination. Such marked variations usually are not observed with respect to pollen seasons.

5. For the proper evaluation of the etiological, diagnostic, and therapeutic factors in mold allergy similar extended studies will have to be made in other parts of the country.

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DISCUSSION ON "MOLD FUNGI IN THE ETIOLOGY OF RESPIRATORY ALLERGIC DISEASES. I. A SURVEY OF AIR-BORNE MOLDS" BY DR. MARIE B. MORROW, MR. E. P. LOWE, AND DR. HOMER E. PRINCE;* "THE COMPARATIVE ATOPIC ACTIVITY OF ALTERNARIA SPORES AND MYCELIUM" BY DR. HENRY N. PRATT WITH THE ASSISTANCE OF MISS RUTH CROSSMAN;† "AIR-BORNE FUNGUS SPORES" BY DR. THEODORE B. BERNSTEIN AND DR. SAMUEL M. FEINBERG

DR. FREDERICK W. WITTICH, Minneapolis.—The pioneer mold surveys of Durham, Feinberg, and Little, Prince and Morrow, Schwonwald, MacQuiddy, the Association of Allergists for Mycological Investigation under the guidance of the University of Texas group, and others are assuming considerable significance. The discrepancies of percentage figures in statistical surveys as now conducted are obvious. Local surveys include only those species controlled by the factors conducive to growth of such species in that area. An investigation such as the A. A. M. I. is making, although based on an average of biweekly plate exposures in spite of weekly and monthly variations, assumes greater importance as the length of their survey is conducted.

It is interesting, not knowing the total number of species identified and counted by Drs. Bernstein and Feinberg over a five-year period in downtown Chicago, that 72 per cent of media-grown spores were composed of *Alternaria* and *Hormodendrum*.

The A.A.M.I. group, over a two-year biweekly period through the central United States from the Great Lakes to the Gulf, found that 61 per cent of the total number of molds isolated comprise these two species. *Alternaria* showed 35 per cent, and *Hormodendrum*, 26 per cent. The A.A.M.I. counts for Minneapolis, over a two-year period, totaled 77 per cent for *Alternaria* but showed only a seasonal variation from June to November—but 20 per cent greater than the other six-month period.

*See page 215 for article by Morrow, Lowe, and Prince.

†See page 227 for article by Pratt and Crossman.

It is improbable that deductions can be made entirely from local observations to account for the lack of seasonal variations in the symptoms of patients with clinical mold allergy at the present time. Observations, both clinically and by slide counts the past five years on other definitely proved air-borne molds like grain and grass smuts, as well as rusts, prove the latter to be not only contributing factors, but primary causes of clinical allergy as well.

The question arises, "What is the significance of these counts?" It should be remembered that mold spores and pollen grains are not strictly comparable. The pollen grain is more easily disintegrated and dissolves more quickly, and the pollen-sensitive patient therefore gets a greater amount of protein absorbed. A spore, however, is a complete cell in itself and is hardier in resisting knocks. It dissolves with difficulty, as those who make extracts know, and therefore is absorbed more slowly even in the highly mold-sensitive patient than is the pollen in the pollen-sensitive patient.

It would stand to reason that a higher mold spore count would be required to produce comparable symptoms in patients of equal mold or pollen sensitiveness. It takes about five to ten times as many mold spores as pollen granules. However, when the saturation point of either reaches its maximum, the patient, of course, will show equal maximal responses.

It may be of interest to mention confirmatory evidence of Dr. Pratt's work. In 1934 Dr. Feinberg called attention to the weak skin test responses of mycelial extracts of molds compared with those of the mold spores themselves. I made similar observations in 1938 with corn smut cultures (grown on potato agar) furnished by Professor E. C. Stakman, plant pathologist of the University of Minnesota School of Agriculture. This produces a growth of matted sterile mycelium. The total nitrogen content of the corn smut chlamydospores was 2.68 per cent by weight, with comparable total nitrogen content for the mycelium. No protein nitrogen content determinations were done. Parallel endermal testing with both the chlamydospores and the mycelium were done on four patients considered to be primary corn smut-sensitive cases. Very definite skin responses with the extracts of the chlamydospores were obtained, but only slight responses were obtained with the mycelium extracts.

Standard methods, closer cooperation, as well as systematic local and general surveys of all atmospheric fungi will be necessary if we are to arrive at any definite conclusions of the importance of mold allergy.

Dr. Edna S. Pennington, Nashville.—For some time air-borne molds as allergens have been the subject of much discussion. Such long-continued, detailed, and far-reaching mold surveys as those reported today help to clarify a problem in which accurate and widespread observations are needed. Mold surveys are as important to the mold problem as pollen surveys are to the pollen problem.

Drs. Bernstein and Feinberg's survey of the Chicago area by the daily culturing of the outdoor air carried on for a 5-year period has given us invaluable information as to the daily, seasonal, and annual fluctuations of the mold content of the air in that territory. Similar surveys need to be carried out in widely separated parts of the country.

The work of Drs. Morrow, Lowe, and Prince is unique in that it represents a much needed effort to analyze and compare the mold content of the air by the method of studying cultures of the air from different localities in widely separated parts of the United States. Their survey carried on for two years in eighteen cities, north and south, has given us greatly increased knowledge of the geographic variation as well as the seasonal variation in the number and variety of molds in the air. If their survey is continued over a longer period of time and in even wider territory, their contribution will be even more valuable.

Drs. Morrow, Lowe, and Prince have called attention to the fact that the occurrence of molds in the southern part of the United States is less seasonal than in the northern part. We have found, as they did, that molds occur in considerable quantity in the air in Nashville in the winter months.

On our plates, exposed two or three times weekly to the outdoor air during the winter months, there are few days when we do not obtain from three to a dozen colonies after a ten-minute exposure.

They found, as did Feinberg and Bernstein, that *Alternaria* and *Hormodendrum* made up the largest percentage of the total molds in the air and that this was true more in the summer than the winter. Although over their entire territory they found that *Alternaria* spores occurred in the greatest number, we found *Hormodendrum* to have the highest incidence. This corresponds with their analysis of Nashville air. *Aspergilli* and *Penicillia*, which they found in all their stations, seemed to occur in fewer numbers and showed very little seasonal variation.

Just a word as to the plate and slide method of studying the molds in the air considered in the light of the conclusions drawn from these papers. The plate or cultural method of analyzing the molds in the air is more complete and reliable than the slide method, presumably yielding colonies from all spores which will grow on artificial media in the air passing over the slide. These can be identified quite accurately by a mycologist with adequate time and facilities for this work, and wherever possible this method should certainly be used, at least to supplement and check the slide method.

Only molds whose spores possess a characteristic appearance and suitable size can be identified with the slide method. These include, in our experience, *Alternaria*, *Hormodendrum*, *Helminthosporium*, *Macrosporium*, *Spondylocladium*, *Acrothecium*, *Stemphylium*, *Fusarium*, smut, and possibly one or two others, which can be identified with some degree of certainty.

Nevertheless, the slide method is available to those who treat mold-sensitive patients and do not have access to the necessary facilities for the cultural method.

The papers read this afternoon would indicate that for practical purposes we could identify those molds of the *Dematiaceae* species, which are most abundant in the air (*Alternaria* and *Hormodendrum*), and also others which seem to have a particularly seasonal variation (*Fusarium*, *Stemphylium*, *Acrothecium*, *Helminthosporium*, and *Spondylocladium*), as all the authors this afternoon seem to have agreed. Thus the slide method should furnish a practical and immediately available aid in determining the daily fluctuations of those molds which may be expected to vary most with the seasons and which make up the larger per cent of those dominant in the air.

DR. LEONARD H. HARRIS, Elyria, Ohio.—For the past three years we have had the opportunity of making a survey of the air-borne molds in our vicinity which is a small town not far from Cleveland, and our results might be briefly stated to compare very favorably with those that have been described in the curves shown in the north and in Chicago, in the first and third papers respectively.

Our mold counts are highest in the summer months. Beginning with the month of May they rise rapidly in June and remain high throughout August, September, and October. The counts begin to fall, as a rule, rapidly in November, with the exception that the mold *Hormodendrum* frequently remains high throughout that month.

It might be mentioned and it has possibly been pointed out before that *Hormodendrum*, at least in this vicinity, has two peaks. The first peak occurs in June and thereafter frequently falls, to rise again in October and November. In this vicinity we feel that it is possible that this double rise coincides with the activities of the large numbers of tomato greenhouses in this region.

These greenhouses are between Cleveland, Berea, and Elyria, and they have two crops, one in June and one in the fall months mentioned. At these times an infestation of molds occurs on the tomato leaves. It is possible that this has some influence on the *Hormodendrum* group curve.

Our second important mold is *Alternaria; Botrytis* is third, and there are small numbers of *Penicillium*, and *Aspergillus*, and *Monilia nigra* seen the year round.

We made some counts in Cleveland in connection with Dr. William P. Garver, sporadically, last year, and continuously thus far this year, and we were rather amazed to find two very striking dissimilarities between the counts in Cleveland, and in our smaller community, 25 miles away. First, the counts of *Alternaria* and *Hormodendrum* were much higher in Elyria than they were in Cleveland, while the counts of *Penicillium* and *Aspergillus* were much higher, consistently, in Cleveland than in Elyria.

After hearing these excellent dissertations on the great numbers of molds that are in the air in our various communities, we, as practicing allergists, should finally remember that it has yet to be shown conclusively that molds, other than *Alternaria*, *Hormodendrum*, *Penicillium*, and *Aspergillus*, are definitely of etiological importance in producing clinical allergy.

DR. WARREN T. VAUGHAN, Richmond.—I noticed in Dr. Prince's charts that there was a surprisingly high breakage of Petri dishes. As I understand, each *X* mark indicated a broken dish. We had that same problem and solved it in a way that I should like to suggest for his consideration. You can get flat ointment tins about the shape of and nearly as large as Petri plates. They have cardboard stamped in the lid, on which you can write any data. Possibly some of you know about them, because some of the state boards of health use them for shipping specimens of feces for examination for parasitic ova.

We started out using these tins containing Sabouraud's medium. Now we use much smaller ones, about half the diameter of a Petri plate, and we find them very satisfactory. When we have made our cultures, we throw them away and then use new ones.

DR. FEINBERG.—First of all, I should like to concur wholeheartedly in most of what Dr. Pratt had to say about the difference between spore antigenicity and mycelium antigenicity, that is, from a quantitative standpoint. Those of you who have discussed with me in the last few years the practical question of mold allergy know how much I have emphasized the importance of having plenty of spores on cultures to be used for extracts. For example, I have stressed the necessity for selecting a type or a species or a strain of *Alternaria* which yields a large number of spores and the advisability of looking at an *Alternaria* culture, after continuous subculture, to ascertain whether it had degenerated in its capacity to produce spores abundantly.

Now, all of these points that I have emphasized were not on the basis of casual impressions but have been based on experience we had a few years ago with just such experiments as Dr. Pratt has mentioned. We raised young cultures of *Alternaria* and other molds and obtained very little reactivity with them after extracts were made. Then we used nine or ten different synthetic media containing nitrogen, phosphorus, and other necessary elements from sources other than protein. We were able to raise good, healthy growths of molds, but in the majority of instances they contained very few or no spores at all, and extracts of these contained very little potency as determined by skin reactions on allergic persons. I would go further, from my own experience, to say that the mycelium did not contain 0.01 or perhaps 0.001 part of the potency of the spores.

There is one point in Dr. Pratt's paper on which I should like to comment; that is, the question of cross-neutralization or cross-desensitization of these spores and mycelium. I believe, if I understand correctly, that he interprets his findings to the effect that the spores contained antigen which neutralized the reagins of mycelium, but not vice versa. If he has made that conclusion, I believe it is open to criticism, because one is dealing here with entirely different degrees of potency. That is a criticism which, as I see it, pervades a great deal of the work on desensitization and cross-desensitization experiments.

Before one can draw conclusions as to whether one is dealing with two similar antigens of entirely different strength, one has to make biologic standardization, by obtaining first comparably reacting extracts. It makes no difference whether it is 1:100,000 or 1:100, but, once one determines the dilutions of the antigens giving equal reaction, then he can make comparable desensitization by the passive transfer method. From the experiments cited by Dr. Pratt, I could conclude only that the spore antigen is much more powerful than the mycelium but not that it is a different antigen.

In regard to our own paper, I want to emphasize one point, namely, as Dr. Pennington and others have said, *Alternaria* and *Hormodendrum* are still the most important fungi from the standpoint of prevalence, at least in a great part of the country. That is a practical point to bear in mind.

Another point I wish to stress is that there is a great tendency to treat patients with mixtures of mold antigen, the relative amounts of the component fungi being based on the degree of skin reactivity. For instance, including in the treatment mixture a large proportion of *Aspergillus* extract because there has been a four-plus reaction to this fungus has no practical or scientific justification. I believe that, when

making mixtures of any kind of antigens, whether pollen or fungi, and in adjusting the relative proportions, one should pay less attention to the degree of reaction and more attention to the degree of exposure of persons to that particular antigen.

DR. B. GELFAND EFRON, New Orleans.—Mention was made of biologic standardization of extracts as it refers to fungus extracts. Biologic standardization should be done for all types of extracts. The validity of skin tests, whether direct or passive transfer, depends upon the use of nonirritant concentrations of extracts. By nonirritant concentrations of extracts we mean those which produce significantly fewer positive skin reactions in control series than in series of patients whose allergic symptoms are attributable to the particular substance in question.

For the sake of discussion, let us assume that we have a 1:5,000 dilution of *Alternaria* extract. Our first step in determining whether this concentration is or is not irritant is to test it on a series of control patients. By a control patient we mean a patient who has no symptoms attributable to allergic disease. We test our extract on 100 or 200 control patients and determine the incidence of positive reactions it produces. Let us assume that our 1:5,000 concentration of *Alternaria* extract produced positive reactions in twenty of 100 control patients. Our next step is to test a series of patients whose presumably allergic symptoms could be due to sensitivity to *Alternaria*. Let us assume that seventy-six of 100 such cases reacted to the extract.

We are now ready to determine whether our extract produced a significantly smaller incidence of positive reactions in control than in allergic cases. The data in our example may be analyzed by the method of the difference between two proportions or by means of the Chi-square test. Analysis by either method shows that the difference is significant, and, hence, we may conclude that our extract is nonirritant. However, if the difference in incidence of positive reactions in control and allergic series was found to be not significant, then our extract was irritant, and we could draw no conclusions concerning the nature of the skin reactions.

We have been publishing a series of communications in the International Correspondence Club of Allergy on Biologic Analysis in Allergy. This series of articles briefly discusses the statistical methods involved in the biologic analysis of allergenic extracts.

DR. PRINCE.—Dr. Harris' remarks about the higher concentration of *Hormodendrum* in the smaller rural district in which he lives are interesting. Such differences might be explained by local variations in habitat, such as the plants that he mentioned forming the natural host for the *Hormodendrum* genus.

It is gratifying that our data secured from exposures as infrequent as twice per month have paralleled in essential points the figures obtained by Dr. Bernstein and Dr. Feinberg. We regret that our facilities did not permit exposure of plates more frequently. In the beginning we made exposures every five days but immediately had to discontinue such frequent observations because our facilities were quickly overwhelmed. As we mentioned at the outset of our paper, we wanted only qualitative data and we felt that this could be obtained on the less frequent exposures.

We are at this time enrolling a new station in Boston where we are exposing plates more frequently. We plan to compare the data obtained from more frequent exposures at this station with data selected at semimonthly intervals. We expect to get some difference in the figure compilations; just how much difference awaits to be seen.

DR. PRATT.—Dr. Feinberg, I believe your criticism is justifiable. We do cross-neutralization because at the moment it is the best method at our disposal. The practical application of the results to the making of extracts, I believe, still stands.

Dr. Efron, I may say that our extracts were completely nonirritating in all normal persons on whom they were tried and on a high percentage of allergic persons who were tested routinely with the extracts.

If I may digress for a moment, I should like to make one or two remarks in relation to the other two papers. In Boston we have made daily exposures of plates for three years, and our results agree almost perfectly with those of Dr. Bernstein and Dr. Feinberg, even to the percentage of the more common molds in the air. I believe the figures would be very, very close if we were to compare them. Both cities are rather northern cities, Boston and Chicago, and I see no reason why they should not be similar qualitatively. I am not sure they are alike quantitatively. Dr. Feinberg uses considerably larger plates than we use.

There were some remarks made in relation to the small size of mold spores, I believe by Dr. Wittich, and that for that reason they might not be so important as exciters to allergy. Our figures show that approximately 10 per cent of seasonal cases among children are sensitive to molds.

The small size of spores and their irregular shapes mean that they settle out of the air very much more slowly than the smooth pollen granule. The chart shown by Mr. Durham on Dr. Cocke's work shows that the number of granules of ragweed, per cubic yard of air even when the small size is taken into account, is greater than calculated. Dr. Cocke attributes this to the spiculated surface. I am inclined to believe that mold spores, with their small sizes and irregular surfaces, contaminate the air much more than the gravity method would indicate.

DR. BERNSTEIN.—The symposium on fungi has been so thoroughly discussed by Dr. Pratt, and Dr. Prince, and the various discussants, including Dr. Efron, that I do not have many more remarks to make. However, it is quite encouraging to see that various surveys have been carried on in different localities by different investigators, each one working independently of the other, and that the data derived from these surveys agree so completely as to the incidence of the types of fungi, their percentage distribution, and their seasonal variations.

Dr. Prince has explained the statement of Dr. Harris regarding the higher incidence of *Hormodendrum* and *Alternaria* as compared with Cleveland. However, the reverse observation concerning the higher incidence of *Aspergillus* and *Penicillium* in the Cleveland vicinity cannot be explained on the basis of our present knowledge.

INFLUENCE OF ACUTE INFECTION ON THE PLASMA LIPIDS AND THE COURSE OF ECZEMA

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FOR the past decade there has been a great deal of interest in the probable relationship between plasma lipids and the etiology of skin diseases. In 1929, Burr and Burr[1] discovered that rats placed on completely fat-free diets which were adequate in all other respects soon developed a definite syndrome characterized in the early stages by scaliness of the skin and in the later stages by necrosis of the tail. Burr[2] observed that, when certain fats were added to the diets of these animals, their general condition improved and the skin scaliness disappeared. He later found that the essential substances were the unsaturated fatty acids, such as linoleic and linolenic, in fat. Turpeinen[3] added arachidonic acid.

Hansen[4] considered a clinical application for the observation of Burr. He began by giving fats with a relatively high unsaturated fatty acid content to infants with eczema. Corn oil, raw linseed oil, and later lard were employed. Some improvement was noted. An effort was made to determine whether any relationship might exist between the rats suffering from unsaturated fatty acid deficiency and the infants with eczema. Blood studies were obtained for the animals and the infants. It was found that in both groups the degree of unsaturation of the fatty acids in the blood as measured by the level of iodine absorption was lower than normal, indicating that the unsaturated fatty acids must be given serious consideration as an etiological factor in infantile eczema.[4, 5]

Stoesser and McQuarrie[6] meanwhile observed that in infants and children there was a definite lowering of the levels for the plasma lipids at the height of acute infections as compared with the values obtained during convalescence. Diet had no demonstrable influence. Fever per se was not responsible for the lowering of the various lipid constituents of the blood plasma. Further studies revealed that the lower levels for the total fatty acids were accompanied by a reduction in the number of unsaturated fatty acids as indicated by subnormal iodine numbers.[7] The already moderately low levels for the serum unsaturated fatty acids in the eczematous infants were still more re-

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duced by acute respiratory diseases, and there was a tendency for them to remain below the normal range for some time.[8]

In spite of the statements of a few clinicians to the contrary, there is still a strong feeling that infants and young children with eczema are very susceptible to acute infections, especially of the respiratory tract. Physicians have differed in their reports on the influence of these infections in children; some observers have stated that there has been improvement of the eczema in the presence of infections, and others stated that the allergic condition became worse. With the evidence that cases of eczema tend to have abnormally low serum unsaturated fatty acids and that improvement followed the ingestion of fats which usually raise the level of the fatty acids, acute infections with their depressing effect on the unsaturated fatty acids should lead to an aggravation of the eczema with slow recovery. Is this what takes place in each case? To answer this question, the decision was made to observe very closely each infant admitted to the hospital for the treatment of eczema. During the winter months, a number of these infants developed acute respiratory infections. This afforded an excellent opportunity to make an accurate study of the influence of infections on the course of the allergic disease.

MATERIALS AND METHODS

Nine infants ranging in age from 5.5 to 16 months were chosen and placed under constant environmental conditions in the hospital. Nurses, who were carefully instructed in the care of these patients, weighed them daily, bathed them with mineral oil, and applied only one ointment, namely, *unguentum acidi borici*. To prevent scratching, restraints were employed, and occasionally it was necessary to use sedatives, such as chloral hydrate and phenobarbital. The diet was kept as constant as possible in all subjects. An evaporated milk formula consisting of equal parts of irradiated unsweetened evaporated milk and distilled water with 7 per cent dextrimaltose was given. A variety of the precooked cereals together with plenty of vegetables and fruits was offered to the children. The only food which was restricted was egg. It was eliminated completely from the diets.

The nine eczematous infants had acute infections of the respiratory tract with elevated temperatures. The fever was high in some of the cases. During the febrile period of the illness, the subjects did not take as much food as during convalescence. Little medication was given. The administration of fluid or blood subcutaneously, intramuscularly, or intravenously was not permitted. The first sample of blood to determine the degree of unsaturation of the total fatty acids was obtained shortly after the admission of the patients to the hospital. Subsequent specimens were drawn at the height of the acute infections which these infants developed and during the convalescent periods.

The time at which each sample of blood was collected was recorded with the rectal temperature and the white blood corpuscle count. In the majority of the cases determinations of the hemoglobin content were also made in order to check the factor of blood concentration. As far as possible the blood specimens were obtained under identical conditions in regard to the time of day and relation to meals. The majority of the samples were drawn between twelve and sixteen hours after a meal.

The blood was collected in specially cleaned tubes, and as soon as possible the plasma was separated from the cells by centrifugation. The plasma was quickly poured into an alcohol-ether mixture, and the various chemical analyses were made. Bloor's[9] methods were followed to determine the blood lipids. The Rosenmund-Kuhnhenn[10] method as modified by Page, Pasternak, and Burt[11] was used to determine the iodine absorption of the serum fatty acids.

The type of disease was practically the same in each case. All of the infants had positive family histories for allergic diseases. All were fed artificially in early infancy. The dental tests revealed egg white sensitivity, and a few patients also had positive reactions to cow's milk and some of the cereals. The distribution of the eczema was more or less uniform in all of the children. There was little or no seborrhea of the scalp. The eruption was confined chiefly to the face and upper trunk and to a lesser extent to the extremities. The plan was to observe only those cases which could be classified as the "true eczema of Moro."[12] All of the subjects were carefully inspected three times each day with the nurses in attendance, and detailed descriptions were made on special record forms. From this information, the course of the eczema could easily be followed and represented in graphic form on charts. The relative heights of the columns revealed the severity of the eczema and gave a good picture of the relationship between the changes in the condition of the skin, the various phases of the acute infections, and the fluctuations in the unsaturated fatty acids as shown by the iodine numbers. The word "tar" was placed toward the end of some of the periods of observation to indicate that 1 per cent crude coal tar in zinc paste had been substituted temporarily for the boric acid ointment.

RESULTS

An examination of the charts in their natural order revealed most interesting data concerning each case. Fig. 1 presents the first three infants who were studied.

CASE REPORTS

CASE 1.—J. S. was a 6-month-old boy who was admitted to the hospital with rather severe eczema of the face. The skin of the chest and arms was moderately involved. The patient was permitted to become adjusted to the routine care of

the hospital for a few days, and then the first blood specimen was drawn to determine the status of the unsaturated fatty acids. The iodine number was 90, which was more or less consistent with the studies of Hansen, who found that in the majority of the eczematous infants the iodine numbers were below 100 before any special fat therapy had been started. Less than a week after admission, the infant began to show signs of an upper respiratory infection and this was almost immediately accompanied by a rise in temperature. During the early part of the febrile period, the eczema improved markedly, but in a few days a change took place. The skin condition became much worse, and the child was restless. Another blood sample was obtained, and the iodine number was now down to 81. After eight days of the illness, the temperature dropped and the infant was more quiet. The eczema began to clear up gradually. Improvement continued, and at the end of the period of convalescence the third specimen was collected. Now the iodine number had risen to 97. The patient was occasionally scratching and traumatizing his skin and was considerably relieved by the application of the tar ointment. There was no further increase in iodine number during the period of observation.

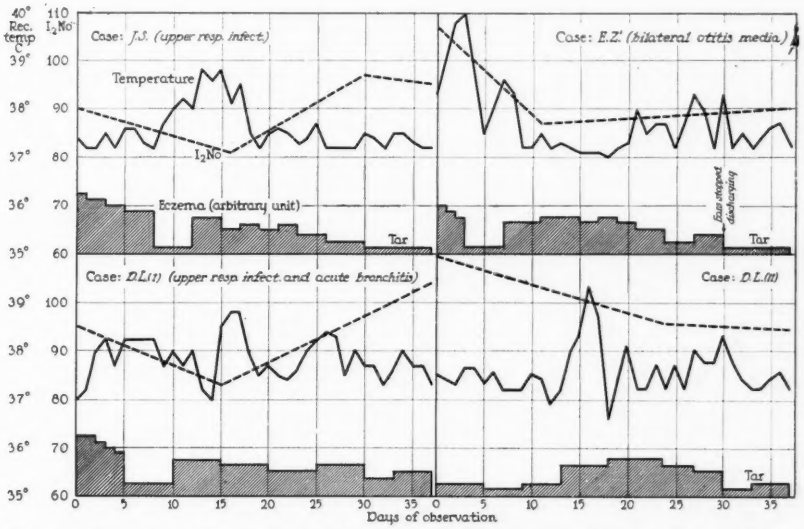

Fig. 1.—Four periods of observation on the first three cases to be studied are presented. Emphasis is placed on the temperature in order to indicate the presence of an acute infection by fever. The changes in the iodine number of serum fatty acids are revealed together with a graphic picture of the severity of the eczema; the worse the condition of the skin, the higher each column.

CASE 2.—E. Z., a 5.5-month-old boy, had an extensive eczema of the face, trunk, arms, and lower extremities. A short time after admission to the hospital, the infant was found to have an acute infection of both middle ears. A blood specimen was immediately drawn, and the iodine number of the serum fatty acids was found to be 107. The eczema began to improve rapidly during the first half of the febrile period, but at the end of this period the condition of the skin became worse. The iodine number had fallen to 86. Rupture of the eardrums occurred, and the temperature dropped to within the normal range, remaining there for ten days. Little change appeared in the eczema. Then a secondary rise in temperature took place, and the skin began to clear up rapidly. Finally the ears stopped discharging pus, and the patient appeared to be well. The iodine number had slowly risen to 90. Tar ointment was applied, and the eczema appeared to be under control.

CASE 3.—D. L. was a girl 6 months of age. She was admitted to the hospital with a weeping eczema of the face and extremities. Soon after admission a blood sample was obtained, and the iodine number of fatty acids was found to be 95. In a few days the infant's temperature began to rise, and it was observed that she had an upper respiratory infection. Again, as in the other cases, there was a period of improvement in the skin disease during the early stages of the infection. As the fever continued, the eczema became worse and the iodine number dropped to 83. Convalescence was marked by short periods of moderate elevations in the temperature. Improvement in the condition of the skin was slow, but at the end of the period of study for the subject the skin was in better shape than at time of admission. The iodine number had gone up to 105.

D. L. remained in the hospital, since the mother was unable to come and take her home. The respiratory disease disappeared entirely, and the eczema continued to clear up. It was thought to be worth while to observe the patient for another month so that we might catch any recurrence of an infection of the respiratory tract. The first step was to collect a blood sample for a determination of the degree of unsaturation of the fatty acids. The iodine number was found to be 111. Twelve days later a sudden rise in temperature occurred. This time there was an infection of the larynx which was followed by an extension of the disease to the bronchi. The child was uncomfortable, and sedatives were necessary. No further improvement took place in the skin condition during the early stages of the febrile period. On the contrary, the eczema became much worse as the irritability of the patient increased because of the coughing. The iodine number dropped to 95 and continued to fall slowly during convalescence. The infant still harbored some infection, and as a result the temperature was more or less irregular until near the end of the period of observation. The tar ointment was then applied to hasten the healing of the skin, and the child was discharged from the hospital in order to reduce the chances of developing another respiratory tract disease.

Fig. 2 shows the data of a second group of infants who were subjected to periods of very close observation during their stay in the hospital.

CASE 4.—L. M., an 8-month-old boy, was admitted because the eczema on his face and arms had refused to respond to treatment in the child's home. The iodine number of serum fatty acids was 96. In the hospital the skin condition began to clear up slowly until the seventh hospital day, at which time there was a slight rise in temperature with a definite improvement in the eczema. The iodine number had risen to 100. A few days later the infant developed a high fever, and on examination he was found to have an ear infection. The child became irritable, and the condition of the skin was much worse. The iodine number dropped to 84, and, as the otitis media continued with fever, there was a further loss in unsaturated fatty acids, for the next blood specimen revealed an iodine number of 80. In two weeks, the infection began to subside, and the eczema gradually cleared up. The serum unsaturated fatty acids reappeared, and early in convalescence the iodine number was already 105. At the end of the period of observation, it was up to 110, and the eczema was much better. In fact the skin had not been in such good condition for many months.

CASE 5.—R. S. was an 8-month-old boy whose eczema began to improve shortly after admission to the hospital. The iodine number of the serum fatty acids was 105. One week later there was a sudden onset of high fever, and both ears were found to be infected. The eczema continued to improve, but toward the middle of the febrile period it rapidly became worse. The iodine number had fallen to 78.

During convalescence there was very little improvement in the condition of the skin until there was again a sudden rise in the temperature due to furunculosis. However, the favorable response was only temporary. The unsaturated fatty acids were returning very slowly, for three weeks after the low value of 78 had been reached the iodine number was no higher than 88.

CASE 6.—H. D. was a 7-month-old boy who developed a rather severe infection of the middle ear which was followed by an attack of acute bronchitis. The skin of this patient was in fairly good condition, the eczema being confined chiefly to his face. It remained clear during the first part of the febrile period, but at the height of this period it suddenly began to spread and to become much worse. The

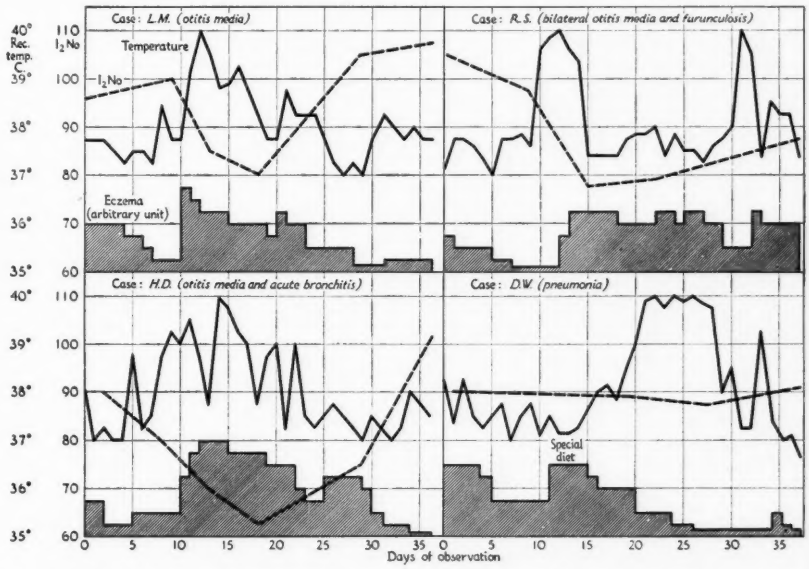

Fig. 2.—The data for the second group of four patients, each of which was subjected to a period of close observation, are shown. The course of the temperature, the variation in the iodine number of serum fatty acids, and the amount of eczema are clearly revealed in each case.

iodine number which had been 90 shortly after admission of the child to the hospital was now down to 62, indicating a rather marked loss in serum unsaturated fatty acids. Early in convalescence there was little improvement, but toward the end the eczema began to heal with rapidity. In a few weeks the skin was in excellent condition. The iodine number had risen to 102 because of the fact that the unsaturated fatty acids were rapidly returning to the blood.

CASE 7.—D. W. was a little girl who was 1 year old at the time of admission. She had a long history of eczema. Practically her entire body was involved by the disease. Various forms of treatment had failed to give satisfactory results. The iodine number was 90. After one week in the hospital, the skin condition appeared to be better, but soon after this the eczema was again worse in spite of the fact that the child had been placed on a special diet from which egg, milk, and wheat were being omitted. Then she developed a fever. The temperature rose rather sharply to a high level, and examination of the patient revealed pneumonia. The skin condition began to clear up rapidly. The improvement extended throughout the remainder of the febrile period into convalescence. The

iodine number underwent little change, although there was some indication that it would rise as convalescence continued.

Fig. 3 reveals the graphic records of the two most interesting cases.

CASE 8.—P. M. was a 10-month-old boy who had been receiving 1 teaspoonful of raw linseed oil three times a day for some time before coming to the hospital. Although the oil had not been given regularly by the parents, nevertheless, the impression was that the amount which the child did receive was sufficient to keep his eczema under control. In fact the patient had just had an attack of acute pharyngitis at home, and there had been no change in the skin condition. The level of unsaturated fatty acids in the blood was fairly good, for the iodine number was 98.

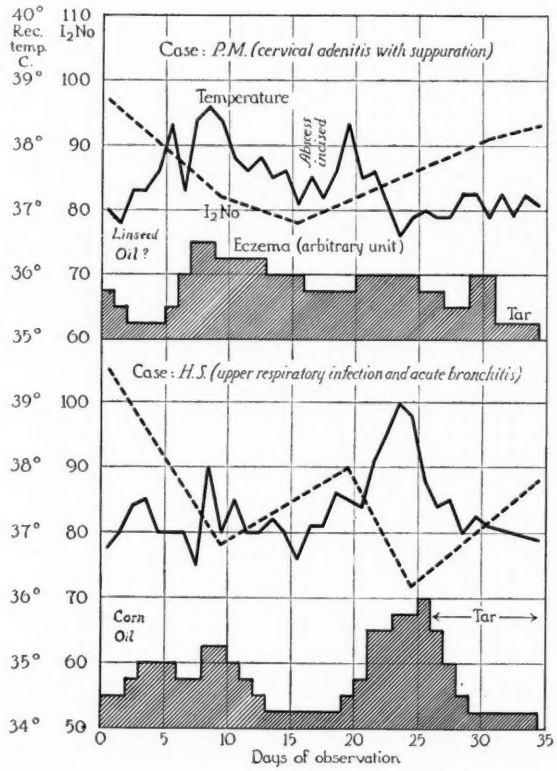

Fig. 3.—The periods of observation on the last two subjects to be studied are presented. The temperature fluctuations, the fall and rise of the iodine number indicating the degree of saturation and of unsaturation of serum fatty acids, and the changes in the eczema are all grouped together for comparison.

A few days after admission the glands of the neck began to enlarge, and there was a rise in temperature. The eczema showed signs of improvement, but this lasted for only a short time, for at the peak of the febrile period the condition of the skin was poor. There was much weeping and trauma from scratching. The iodine number had fallen to 82. The gland infection on the left side of the neck went on to suppuration requiring incision of the abscess. The eczema had remained the same with the iodine number down to 78. The temperature dropped, and convalescence was accompanied by some improvement in the skin condition with a corresponding rise in the unsaturated fatty acids yielding an iodine number

of 90. However, there was still a great deal of scratching, and the tar ointment was employed to help control this. Immediately, the skin appeared much better, and there was a further increase in the unsaturated fatty acids. Throughout the entire period of observation this subject continued to receive the raw linseed oil although occasionally it was not retained.

CASE 9.—H. S. was the last patient studied. This subject, a 16-month-old boy, had had eczema since early infancy, and the mother claimed that he also had short spells of wheezing. Of the many forms of treatment which had been tried, none had met with success although some of the procedures recommended might have yielded satisfactory results had there been good cooperation on the part of the parents. For several weeks before admission to the hospital the boy had been receiving at least 2 teaspoonfuls of corn oil three times a day. Hospitalization was suggested in order to observe whether the patient had bronchial asthma.

The skin was in fairly good condition on admission. The iodine number was 105. On the seventh hospital day there was a rise in temperature associated with the onset of an acute infection of the nose and pharynx. Almost immediately there was some improvement in the eczema, but this was followed by a flare-up in spite of the fact that the corn oil was being administered. The iodine number fell to 78. Recovery from the upper respiratory disease was rapid, and during convalescence the skin condition improved satisfactorily with a rise in the unsaturated fatty acids so that the iodine number was 90.

Unfortunately this appeared to be the prodromal stage of another infection. The temperature again rose, and the patient was found to have a typical attack of acute bronchitis with no evidence of asthma. At the height of this infection the child was very restless. The eczema was worse, and the serum fatty acids became less unsaturated with a drop in the iodine number to 72. Because the skin was badly traumatized by the child's scratching, tar ointment was applied for the relief of itching and to promote some healing. The corn oil was still being taken by the patient. Convalescence was uneventful. The skin condition cleared up rapidly, and the iodine number rose to 88. The child was discharged, and the parents received instructions to continue the oral administration of the oil and the application of the ointment as consistently as possible.

COMMENT

Although the foregoing results cannot help but indicate that there is an important relationship between lipid metabolism, acute infections, and infantile eczema, a word of explanation is necessary. All of the cases of eczema were very much alike. Their nutritional state was good, and they were closely watched in order to avoid any marked changes. The small losses in weight which occurred in some of the subjects during the period of infection were promptly regained. Low lipid values were not due to poor nutrition, as was observed by Man and Gildea[13] in a series of cases. The determinations of the hemoglobin content of the blood which were made at the time the blood specimens were drawn for study revealed that there was not much variation in any one instance, the hemoglobin values being so constant that changes in the blood concentration were considered to play little or no part in the results obtained.[14] The leucocyte counts varied inversely with the total fatty acid

content of the blood plasma. The extremely high counts occurring at the height of some of the acute infections were not all associated with the lowest iodine absorption values.

The iodine numbers of the serum fatty acids obtained in each infant shortly after admission to the hospital showed that the acids were less unsaturated in the more severe cases of eczema than in the subjects with milder skin conditions which were practically under control. This confirms the observations of Hansen.[15] However, Faber and Roberts[16] also made studies concerning the unsaturated fatty acids in eczema and concluded that the low unsaturation of fatty acids in this skin disease is of secondary or incidental etiological significance. Cornbleet[17] thought that an altered lipid metabolism occurred in eczema, and he administered maize oil (unsaturated fatty acids) for long periods of time with a favorable response. Taub and Zakon[18] did not agree. They were upheld by Epstein and Glick,[19] who observed only four cases of infantile eczema. Two of these patients were given raw linseed oil without any improvement attributable to the therapy. Ginsberg and Bernstein[20] admitted to the hospital and studied six infants with eczema. The subjects received either cod-liver or corn oil. Four of them responded favorably; the remaining two showed no remarkable change.

The significance of the unsaturation of fat and the value of therapy designed to increase the degree of unsaturation of the serum fatty acids in infantile eczema apparently have not been ascertained. However, some light might be thrown on these problems by the studies involving the influence of acute infections on the plasma lipids in the infants with eczema. It was observed that at the beginning of the acute illness the condition of the skin improved remarkably although no special treatment was instituted. This period of improvement may coincide with sudden flood of unsaturated fatty acids into the blood stream which Boyd[21] claims occurs with the onset of fever. The case of L. M. (Fig. 2) illustrates this clearly. The same thing may have occurred in most of the other patients, but it was not noted because samples could not be drawn frequently enough without removing too much blood, thereby jeopardizing the life of the patient.

There was one exception. D. L. (Fig. 1) failed to have any change in the eczema at the onset of the fever which accompanied the attack of bronchitis in the second period of observation. This was probably due to the fact that the skin was already in fairly good condition before the acute illness. Any increase in unsaturated fatty acids might therefore not have had much influence, but a loss did have an effect, for during the febrile period the eczema became worse. The iodine number of the serum fatty acids had dropped.

Enough specimens of blood were obtained during the height of the infection in each infant in order to show that there was a definite loss

in unsaturated fatty acids. The already moderately low iodine numbers of the serum fatty acids were still more reduced. No constant relationship existed between the height of the fever or the severity of clinical signs and the degree of saturation of the total fatty acids. The condition of the skin became much worse.[22] Improvement followed in the convalescent period as soon as the plasma lipids had risen to within or close to the normal range and the unsaturated fatty acids had returned to higher levels.

The one exception was Case D. W. (Fig. 2). She had more or less a generalized eczema on admission to the hospital. The skin failed to respond to any form of treatment. The patient developed a rather severe pneumonia with a high fever. This lasted eight days since chemotherapy was not employed. The eczema began to improve rapidly soon after the onset of the infection, and the skin remained in good condition throughout the febrile period and into convalescence.

This case brings up the question as to whether fever per se has anything to do with the improvement in the eczema. The skin of some of the infants was in better condition at the end of convalescence than before the onset of the acute infection. In those cases there was an increase in the unsaturated fatty acids during the convalescent period which was greater than the loss which had been sustained at the height of illness. Fever probably could not have produced this effect, for McQuarrie and Stoesser[23] have shown more or less conclusively that it has little or no influence on the plasma lipids. However, fever could have an effect on the eczema as a form of shock therapy.[24] There would then be little or no change in the serum fats including the unsaturated fatty acids. This may have occurred in Case D. W., for the iodine numbers were practically the same over the entire period of observation.

There appears to be no doubt of the fact that in infants and young children the intensity of the fat metabolism during acute infections, especially of the respiratory tract, leads to a reduction in the plasma lipids with an ultimate loss of unsaturated fatty acids. The change can take place even if the patient is taking a diet containing fats high in unsaturated fatty acids, and it can also occur in infants with eczema even though their serum unsaturated fatty acids may be already below the normal range. In the latter case the degree of unsaturation of the fatty acids can become extensive, and the present studies appear to show that this is associated with a definite aggravation of the eczema regardless of the form of treatment being employed. But is the disturbed lipid metabolism the cause of the unfavorable change in the skin condition or are the bacterial toxins, or even the fever responsible? Further investigations are indicated.

SUMMARY

1. Rats placed on a diet free of certain unsaturated fatty acids develop in the early stages scaliness of the skin and in the later stages necrosis of the tail. Returning the essential acids to the food leads to immediate improvement in the skin condition.

2. Infants with eczema may be given plenty of fats (corn or linseed oil) rich in unsaturated fatty acids, and some of these children show improvement.

3. Examination of the plasma lipids of the rats with scaliness of the skin and the infants with eczema reveals that the unsaturated fatty acids are below the average level in the majority of the cases. After the ingestion of the acids, the serum unsaturated fatty acids usually increase to within the normal range.

4. The conclusion has been reached that the low unsaturation of the serum fatty acids in infantile eczema is one of the important causes of the disease.

5. In order to find out the result of a further reduction in unsaturated fatty acids, such as occurs in acute respiratory infections in infants, we subjected nine infants with eczema to periods of careful observation in the hospital. During these periods all infants had an attack of an acute disease. The skin changes were watched closely, and periodic determinations of the plasma lipids were made.

6. The acute infections were accompanied by a short period of improvement in the skin condition at the onset of the disease, but this was followed by a rather rapid aggravation in the eczema. The height of the infection usually marked the peak of the flare-up in the condition of the skin, and this was also the time when the degree of unsaturation of the serum fatty acids was lowest.

7. The assumption, therefore, is that the acute respiratory diseases cause the eczema to become worse through the medium of depressing the serum unsaturated fatty acids to low levels, and that improvement in the skin condition takes place during the convalescent period because there is a corresponding gradual return of the unsaturation of the fatty acids.

8. In some instances the eczema responded much better to therapy after full recovery from the acute illness than before its onset.

9. This has brought up the question of the influence of other factors, such as the fever, on the skin condition and infers that more studies must be carried out before definite conclusions can be drawn.

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DISCUSSION

DR. BRET RATNER, New York City.—I am very glad to have heard Dr. Stoesser's paper because, ever since I read the reports by Burr, Hansen, and McQuarrie on the importance of unsaturated fatty acids, I have made it a practice to give all of my eczema patients unsaturated fatty acids as a routine procedure.

The question of infection in eczema is probably very important because there have been deaths reported in eczema due to superimposed severe infection.

I should like to ask Dr. Stoesser whether he has correlated his findings with studies on leucopenia. As I understand it, there is a redistribution of leucocytes in eczema and the patient, as a result of sequestration of

leucocytes in the eczematous skin, would thereby develop a leucopenia, and that, in and of itself, may account for the lowered resistance.

I should like to say that, since the reports I mention appeared about ten years ago, I am very happy that Dr. Stoesser has re-emphasized the importance of the necessity for unsaturated fatty acids, and I do think that the routine use of mazola oil, cod-liver oil, and probably linseed oil may help us as adjuvants in the treatment of eczema in infancy.

Dr. Stoesser.—We have made no observations as to leucopenia in eczema up to the present time, but we are beginning to include them in our studies. It might be of interest to know that some of the infants with infantile eczema have had a reversal of the usual blood picture or in other words, a low polymorphonuclear neutrophile, leucocyte count, and a high monocyte count. This has remained so until the children have been more or less free of infection or their eczema has practically disappeared. At that time, which is most often when the child is between 18 months and 2 years of age, the tendency is for the polymorphonuclear neutrophile leucocyte count to rise and the monocyte count to fall.

ASTHMA AND PRIMARY CARCINOMA OF THE BRONCHUS

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ROCHESTER, MINN.

THIS report has to do with records of three patients who complained primarily of asthma, but who were found to have an associated primary bronchial malignancy. As far as is known the malignant processes were independent, coincidental conditions, for primary bronchial malignancy does not represent a complication of asthma. Since asthma is a fairly common condition and primary bronchial malignancy no longer is rare, the association of the two conditions may be more frequent than is generally recognized. It is now felt that 10 per cent of all carcinoma are found in the lung, an incidence that represents approximately 15,000 instances of the lesion each year in the United States.

Primary bronchial malignancy occurs among patients of any age and is encountered three times as frequently among males as among females. The onset is insidious, and the symptoms encountered depend upon the situation of the tumor and the degree of obstruction of the bronchi. The more common symptoms include cough, hemoptysis, and expectoration. The sputum at first is mucoid in character and later becomes purulent. The secretions at some time during the patient's illness may be retained and will then produce chills and fever. Not infrequently, physical and roentgenologic evidence of bronchitis, stenosis, or even atelectasis may be found. Pain is a late symptom, as a rule, and usually indicates pleural involvement; it is therefore indicative of a poor prognosis.

The types of malignant processes usually encountered in bronchi are squamous-cell carcinomas or adenocarcinomas. They are usually of a high degree of malignancy. The localized polypoid adenocarcinomas that can be readily reached bronchoscopically can be treated by surgical diathermy and implantation of radon seeds, followed by roentgen therapy. When feasible, pneumonectomy is preferred for all other tumors, and, for those patients for whom such a procedure is not applicable, roentgen therapy may at times be of value.

Abstracts of the data in three cases illustrative of some of the features of concurrent asthma and primary bronchial malignancy follow.

From the Division of Medicine, Mayo Clinic.

Read before the Annual Meeting of the American Society for the Study of Allergy, Cleveland, Ohio, June 2, 1941.

REPORT OF CASES

CASE 1.—A housewife, aged 42 years, was first seen April 22, 1927, at which time she was complaining of metrorrhagia, which proved to be caused by uterine fibromyomas, and asthma which had been present for thirty-four years. Wheezing

Fig. 2.

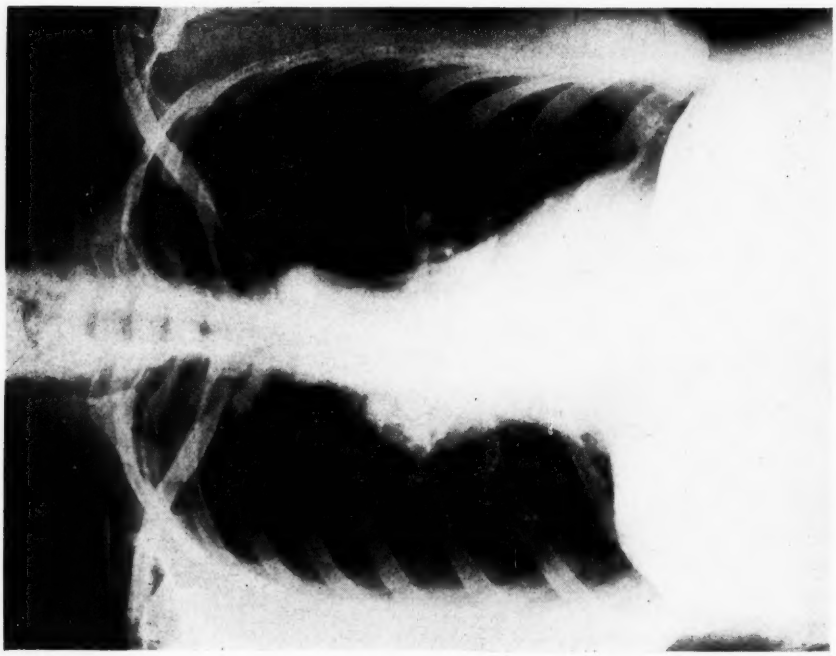

Fig. 1.

Fig. 1.—Roentgenogram of the thorax of the patient in Case 1 made on June 15, 1927.
Fig. 2.—Histopathologic aspects of lesions in the upper portion of the mediastinum in Case 1. Adenocarcinoma of grade 1 (×135).

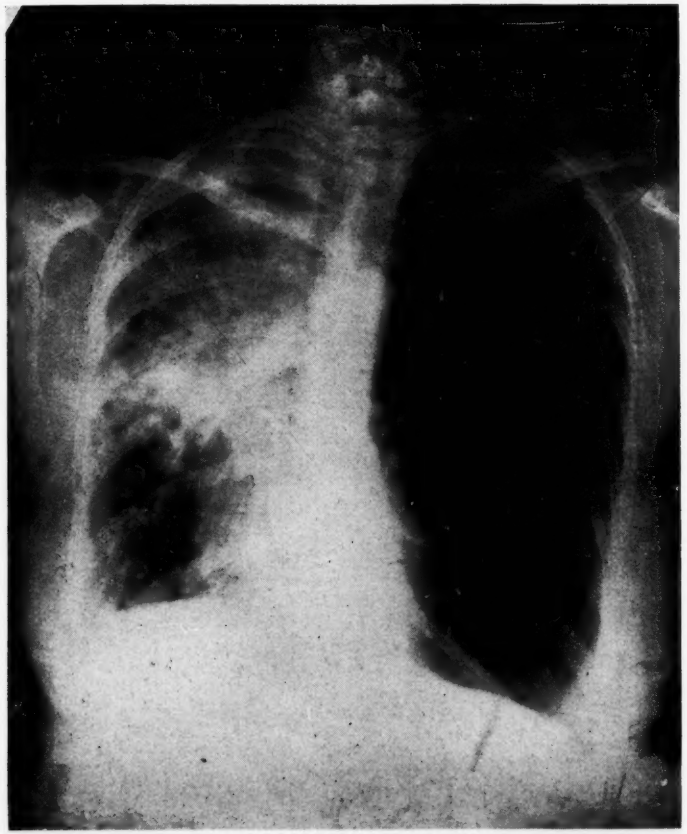

Fig. 3.

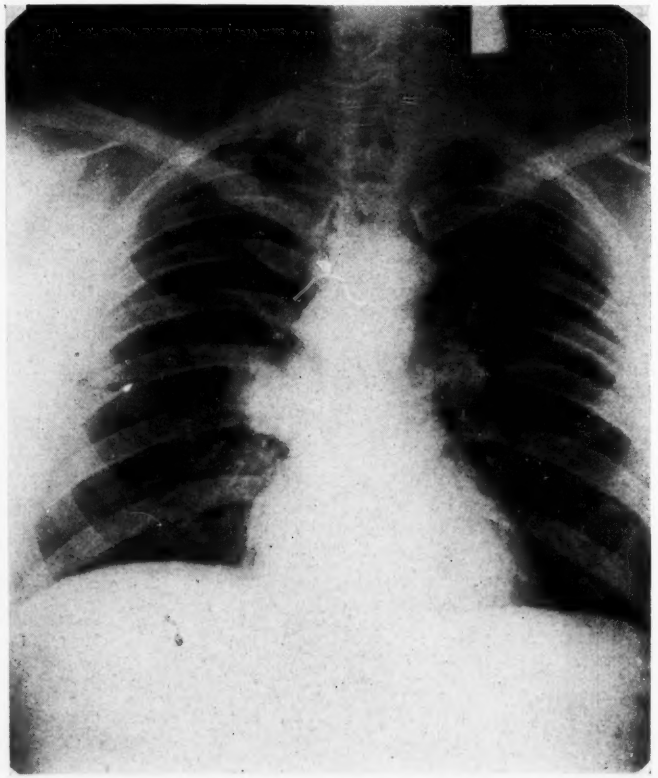

Fig. 4.

Fig. 3.—Roentgenogram of the thorax of the patient in Case 1 made fifteen months after treatment.
Fig. 4.—Roentgenogram of the thorax of the patient in Case 2 made on June 21, 1938.

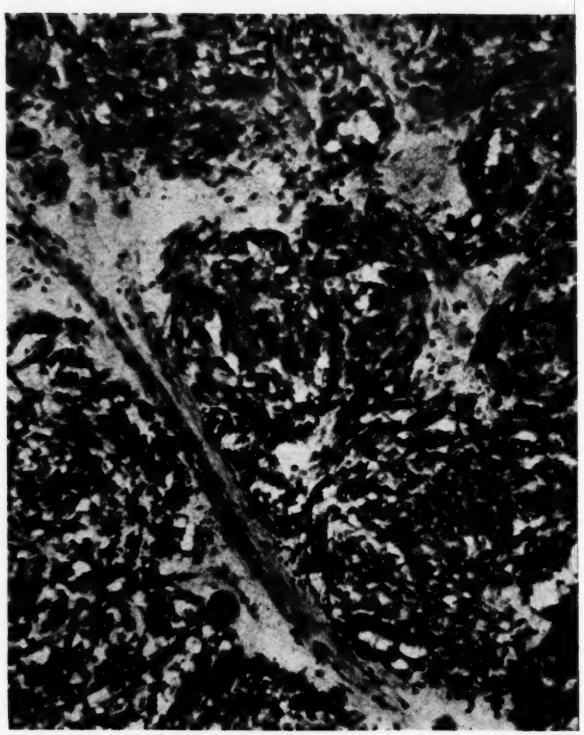

Fig. 5.

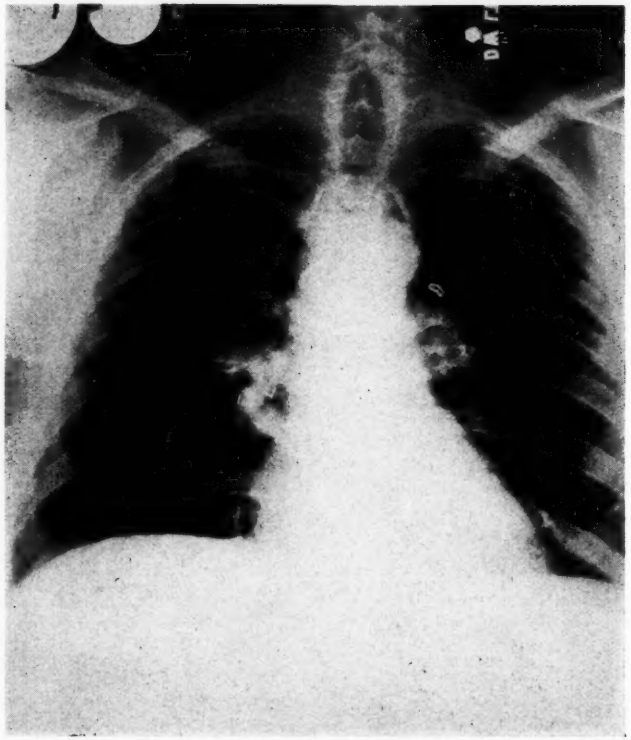

Fig. 6.

Fig. 5.—Adenocarcinoma of grade 4 from right main bronchus of the patient in Case 2 (×135).
Fig. 6.—Roentgenogram of the thorax of the patient in Case 2 made on Sept. 18, 1940.

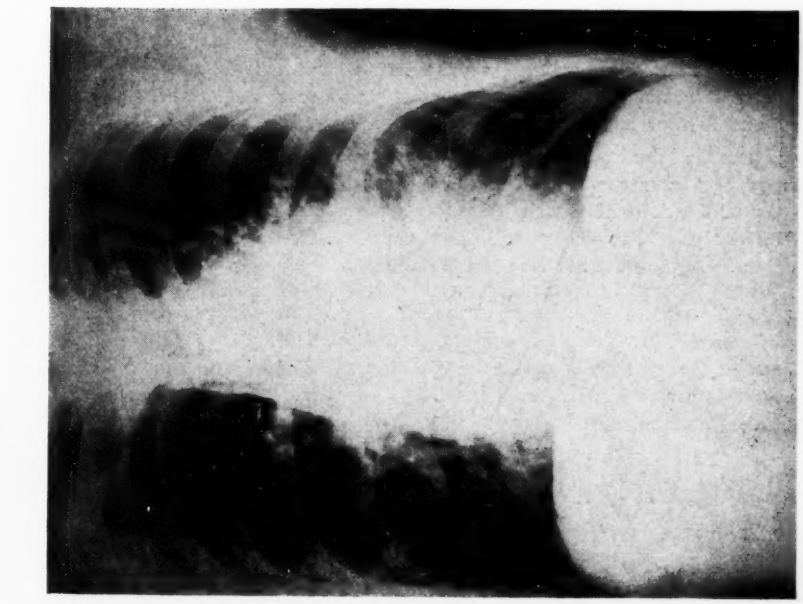

Fig. 8.

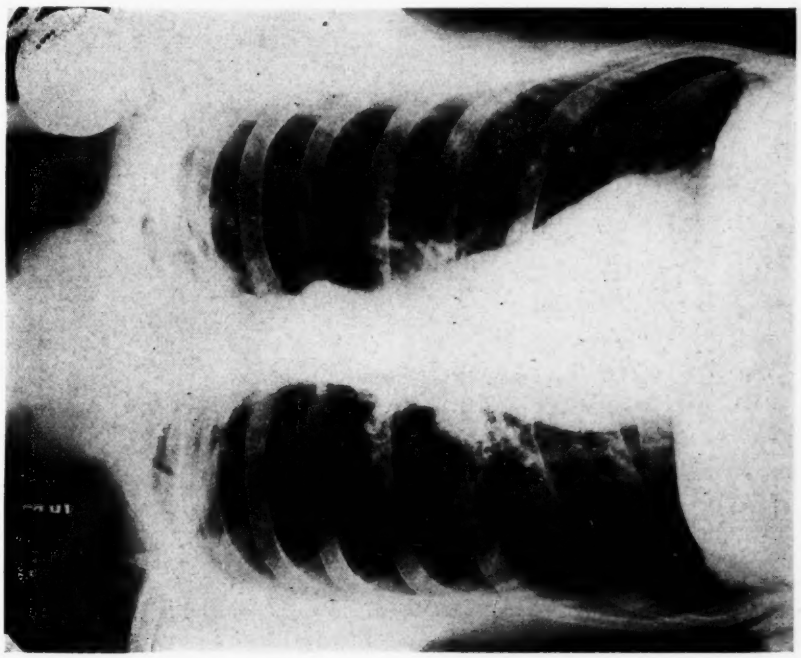

Fig. 7.

Fig. 7.—Roentgenogram of the thorax of the patient in Case 3 made on July 4, 1939.
Fig. 8.—Roentgenogram of the thorax of the patient in Case 3 made in July, 1940.

had become more frequent in the year prior to her visit to the clinic. A roentgenogram of the thorax (Fig. 1) disclosed a circumscribed nodule in the upper part of the mediastinum on the right. Hemoptysis had not been noted, and examination of the thorax resulted only in the hearing of asthmatic râles. Bronchoscopy disclosed an ulcerating, bleeding lesion which filled the right main bronchus, with pus exuding from below. A specimen of the lesion was removed and proved to be adenocarcinoma grade 1 (Fig. 2). Three weeks after roentgen therapy wheezing subsided, and in four more weeks the cough disappeared.

Twelve months later cough, slight hemoptysis, and stridor developed with dyspnea and cyanosis. A roentgenogram made three months later (Fig. 3) showed infiltration of the middle lobe of the right lung and demonstrated that the heart was displaced to the right. In spite of additional roentgen therapy the patient died three months later. A post-mortem examination was not made.

CASE 2.—A man, aged 39 years, was referred to the clinic on June 21, 1938, because of seasonal hay fever and asthma of ten years' duration. There was slight suppression of breath sounds at the base of the right lung, and coarse râles were heard over this area. Four months previously the patient had coughed up a little blood-streaked sputum.

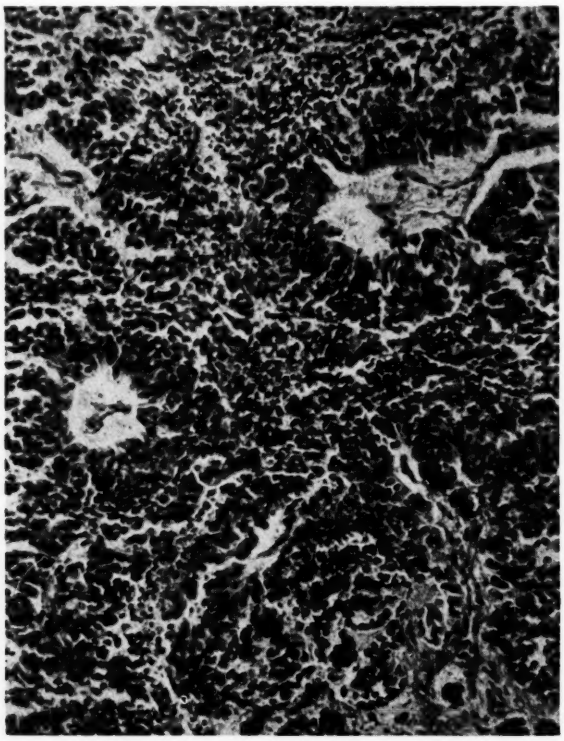

Fig. 9.—Adenocarcinoma of grade 4 from left lung of the patient in Case 3 (×135).

A roentgenogram (Fig. 4) revealed calcified hilar nodes and a circumscribed shadow on the right at the back of the eighth and ninth ribs posteriorly. At bronchoscopy a tumor the size of a dime was found to be almost completely obstructing the right main bronchus. The tumor was removed with forceps and found to be an adenocarcinoma grade 4 (Fig. 5). Radon seeds were inserted into the base of the tumor, and this was followed by roentgen therapy. Yearly re-examina-

tions have revealed no evidence of recurrence to date, although roentgenograms still show some degree of infiltration and the presence of radon seeds (Fig. 6).

CASE 3.—A man, aged 41 years, was first examined at the Mayo Clinic in 1924 because of recurring colds and asthma. In July, 1939, he stated that cough and wheezing had become increasingly severe and that he had had chills and fever at times. Examination showed marked emphysema, scattered moist râles in the lower parts of both lungs and early clubbing of the fingers. A roentgenogram (Fig. 7) showed some evidence of bronchiectasis at the left cardiophrenic angle. Bronchoscopy was advised, but the patient refused because no promise of benefit from this procedure could be made. Symptomatic treatment, including postural drainage, was advised. On July 13, 1940, while he was in Oregon, he complained of a "caged in" sensation in his thorax, with asthma. Râles were heard over the middle and lower lobes of the left lung, and a roentgenogram revealed marked infiltration of the left lung (Fig. 8). Type XIX pneumococci, hemolytic streptococci, and Gram-positive diplococci were found in the sputum. Chemotherapy was ineffectual, and the patient died on Aug. 4, 1940. Necropsy by Dr. Thomas D. Robertson of Portland, Oregon, revealed a friable, cellular tumor mass in the proximal or main left bronchus. Microscopically the tumor was a grade 4 adenocarcinoma (Fig. 9).

COMMENT

These three cases were presented primarily to re-emphasize the importance of the making of a careful and complete examination of all patients who have asthma and the necessity for bronchoscopy in certain cases if the correct diagnosis is to be made.

Recurring hemoptysis among asthmatic patients, although it may be associated with severe cough in asthma, always should suggest the possibility of neoplasm or other organic pulmonary disease. Recurring chills and fever or fever alone complicating asthma suggests the possibility of bronchostenosis with retained secretions, and this condition may be caused by a neoplasm, although more often it is the result of inflammation. Persistent impairment of breath sounds, with or without impairment of the percussion note, justifies bronchoscopy in any case of asthma.

Roentgenologic observation of infiltrating lesions at the hilus pulmonis, even in the absence of a suggestive history or physical observations, also is an indication for bronchoscopy. In fact, at the Mayo Clinic, bronchoscopy is considered in any case of asthma in which the history or physical observations are not typical or in which there is evidence suggesting a stenotic bronchus, for the procedure is easily carried out without risk to the patient.

In addition to the cases reported we have seen a few patients suffering from primary carcinoma of the bronchus, who had been treated for asthma before coming to the clinic. These patients, however, are not included in this group, since the symptoms present actually were not those of asthma. They are mentioned at this point merely to re-emphasize the fact that the symptoms caused by carcinoma of the bronchus in some cases can be and are mistaken for those of asthma.

DISCUSSION

DR. RALPH G. MILLS, Decatur, Ill.—The authors do not assume any etiological relationship between asthma and carcinoma in the cases detailed. The two conditions do occur together occasionally, but apparently not so frequently as one might expect.

If, as estimated, there are about 15,000 cases of carcinoma of the lung developing each year in the United States, and there are about 500,000 cases of asthma (or one case in every 200 of the population), one might well ask why this apparent rarity.

A number of years ago I had occasion to study the subject of lung cancer in some detail from the standpoint of pathology and associated symptoms and was surprised to find asthma so infrequent. It is true that dyspnea from exertion due to purely mechanical obstruction was often found in the later stages of the disease, but true asthma of the paroxysmal type was strangely absent.

Other extensive lesions of the lung also seem to be unrelated to true asthma, and apparently the more extensively the lung tissue is damaged, the less the probability that there will be an associated asthma. Lung abscess of septic or amebic origin, metastatic malignancy, tuberculous destruction and healing, pneumonia, and even silicosis are very rarely associated with true asthma.

All this is in marked contrast with the picture revealed by the lungs in pure asthma in which emphysema is usually the sole gross alteration. In addition a little mucus may appear in the bronchi, but essentially the findings are strikingly slight.

In the presence of this seemingly contradictory situation, one may indulge in a little justifiable speculation.

In the cases cited, the asthma was well established before the malignancy made its appearance. The advent of the complication was not marked by any change in the picture for the worse, and the new element did not alter the group of symptoms to the extent that complications were suspected. And yet retention of secretion must have been present as the malignant growth developed in the bronchi. This is in marked contrast to the situation in which retention of desiccated plugs of mucus in asthma is associated with acute symptoms; there is relief following their expulsion.

Apparently the development of a malignant growth neither causes asthma to appear nor changes its clinical course when its does develop. Its significance then is largely connected with prognosis. The existence of an allergic state in a case of cancer of the lung is seemingly not enough to induce a picture of true asthma. Also extensive tissue destruction alone is not enough to produce it.

It is assumed that histamine forms in the body as a result of tissue disintegration, and it is also assumed that histamine induces muscle spasm in the bronchi, and thus forms the pathogenetic background for asthma. If the growth of cancer, the spread of inflammation, or the development of various infections promotes the liberation of histamine as a product of protein dissolution, then why does it not produce the asthma complex as a feature in every extensive pulmonary lesion?

Retention of secretion within the lung presumably including histamine, such as occurs in unresolved pneumonia, abscess, foreign body,

tuberculosis, and cancer, should increase the probability of an asthmatic attack, whereas free drainage through wide-open bronchi should lessen the probability. Apparently the question of free drainage is not related to asthmogenesis.

Carcinoma of the lung is found more often in those later decades of life in which the so-called intrinsic type of asthma is the more common form. This fact raises the question of a possible bacterial element which cannot be easily dismissed. The influence of a pre-existing chronic bronchitis of allergic nature is to be considered. The ready transition from chronic bronchitis to bronchiectasis with a definite increase in the incidence of concomitant asthma leads one to believe that infection is more potent than malignant transformation.

The question of bronchosinusitis or sinobronchitis is perhaps of even more importance. The extensive pulmonary lesions mentioned above have no influence upon the mucosa of the nose or the accessory nasal sinuses. This fact may suggest a necessary coinvolvement of the two regions if asthma is to result. This condition is definitely bacterial in origin, and it is common knowledge that both clear up when one is treated successfully. Here the amount of tissue destruction is minimal and conforms more closely to the pathology of true asthma.

I have not found a case of malignancy within the nose or its accessory sinuses producing a picture of true asthma. The converse is equally true. The nose is not involved when the lung is even extensively involved. The increase of mediastinal pressure as from tracheobronchial lymphadenopathy or substernal goiter causes neither asthma nor pathologic changes in the nose.

In this discussion there are a number of assumptions that are far from proved. Perhaps the important of these is that histamine is produced in all these conditions mentioned and that its action, if present, is subject to varying intensity. Newer methods of chemical identification of histamine should permit its recognition and assay in the sputum and the various excretions concerned. When this has been accomplished, we shall doubtless be better able to understand the etiology of paroxysmal asthma and the conditions under which it can develop. Meanwhile the associated malignancy in asthma remains as merely a problem relating to diagnosis and its associated prognosis.

If, the coexistence of asthma and bronchogenic carcinoma is more frequent than it now appears, we should seek to emphasize the importance of the association in diagnosis and treatment. If, on the other hand, they do not occur together as often as one would expect, we are called upon to explain that fact. Possibly this explanation may contain a clue as to what asthma really is.

DR. MILTON B. COHEN, Cleveland.—It seems to me that the real importance of this paper lies in the fact that we have defined asthma, for our purposes, too sharply. We think of asthma as bronchial asthma, but, when the patient comes in with signs of asthma, he may have all sorts of things.

I believe we would be very much better off if we would think of asthma as any condition which interferes with the free flow of tidal air as a result of some bronchial obstruction. This can arise from within or without the bronchial tree.

We may have a patient who has some obstruction from without or within, who is not allergic. We should study this patient to determine,

first, whether the obstructive breathing and so-called asthmatic symptoms result from organic change, malignancy, or anything else; and in those who are true bronchial asthmatics, who have complications, we should think in terms not of diagnosis, but of the components which play a part in the interference with breathing.

Unless we do these things, we are going to be criticized frequently by internists and other practitioners, and we are going to make very many mistakes.

DR. MAYTUM.—In answer to Dr. Cohen's remarks, I feel that the diagnosis of asthma should be limited rather rigidly. I believe this is particularly true in cases of cardiac dyspnea. The nocturnal dyspnea seen in cardiac disease is not asthma, and I do not believe it should be called cardiac asthma.

Original Articles

INHALANT SENSITIZATION IN GUINEA PIGS UNDER CONTROLLED ATMOSPHERIC CONDITIONS

I. Description of Air-Conditioned Chamber and Fundamental Principles Underlying Its Use

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Abbie Betts Courtright, B.A.
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THIS paper presents methods for the study of inhalation sensitization in guinea pigs under controlled atmospheric conditions. Since it is desirable to approximate natural sensitization and the resulting symptoms as they occur in man, we constructed a chamber in which varied weather changes can be obtained paralleling those which affect human beings. In our experiments we have used the inhalation method of sensitization perfected by Ratner.[1]

Anaphylactic shock has been studied in the past chiefly by the use of the parenteral route. Busson and Ogata[2] in 1911, and later in 1924 and 1925, however, studied anaphylaxis by the inhalation method. More recently, Sewall,[3] Alexander and his co-workers,[4] Kallos and Pagel,[5] and Preuner[6] have made important contributions to the subject by the use of this method. In 1927 and again in 1939 Ratner[7] published detailed studies on sensitization of guinea pigs by the inhalation of dry horse dander in a closed observation chamber under room conditions.

We agree with him that the inhalation method of sensitization approaches more closely natural sensitization in man. Our reasons for this are as follows: (1) The allergen must enter the body through the nasal and respiratory membrane or be held there[8]; (2) sensitization is built up by repeated exposures which approximate more closely clinical sensitization; (3) the respiratory membranes, because of repeated exposure, may acquire resistance to general sensitization which may not result from the subcutaneous or intraperitoneal routes; (4) the inhalant substance may also be modified, as in nature, by the changing meteorologic conditions; and (5) the respiratory membrane itself may be affected by such weather changes.

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METHODS

CONSTRUCTION OF AIR-CONDITIONED CHAMBER

A diagram of the air-conditioned chamber used in our experiments is found in Fig. 1. The apparatus is divided into two compartments, each 12 by 14 by 18 inches; one supplies cold, dry or cold, moist air, and the other, warm, dry or warm, moist air. The walls of each chamber are well insulated and sealed. A heavy, swinging door containing a thick glass observation window is fitted into each compartment, and a sponge rubber seal makes them airtight when the door is closed. They are equipped with drain pans and wire floor, removable for cleaning.

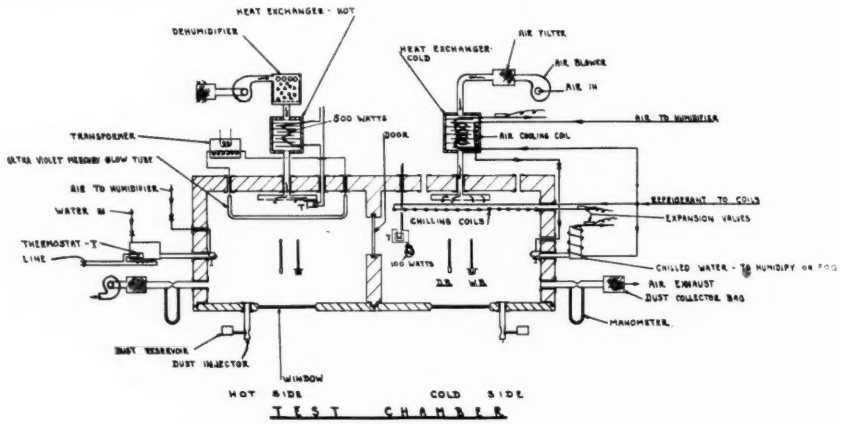

Fig. 1.—Diagram of air-conditioned chamber.

As noted in the diagram, each unit is supplied with wet and dry bulb thermometers and thermostats for temperature control. Chilled or heated water, under pressure, is blown in and regulated as to droplet size (mist, fog, or rain) by a valved nebulizer. Warm or cold air is sucked or blown in through Streit filters to remove most particulate matter from the air. The allergen is pumped in by a powder nebulizer at timed intervals. Water manometers register the air pressure found within the chambers, while the speed of the air blowers and the size of the air orifices control the pressure. With this arrangement it is possible, for example, to expose one group of animals to air at 40° F., 100 per cent R.H., simultaneously with another group at 98° F., 30 per cent R.H. By this arrangement animals can be shifted from one meteorologic condition to the opposite extreme. This shifting simulates the passage of a cold or warm front (Table I).

In the chamber described, barometric pressure could not be shifted to the degree comparable to that found in acute weather changes in the eastern United States. We are, however, planning the construction of an air-conditioned chamber in which barometric pressure will be made to vary from that of 25,000 feet above sea level to that of sea

level. Certain data already recorded would appear to indicate the possible importance of such changes on the normal and sensitized respiratory mucous membranes.[9]

TABLE I*

AVERAGE BEHAVIOR OF THE COMMONLY OBSERVED METEOROLOGICAL ELEMENTS. FRONT PASSAGES OVER THE EASTERN UNITED STATES IN WINTER

ELEMENT	WARM FRONT	COLD FRONT
Pressure	Falling, then steady or falling less rapidly	Falling, then slightly rising, then abrupt rapid rise
Temperature	Steady, then rising at an increasing rate, then steady	Rising slowly, then falling slightly, then dropping abruptly at a rapid rate
Relative humidity	Fairly constant, then increasing to near saturation, then falling	Increasing gradually to almost saturation, then falling
Specific humidity	Steady, then increasing at an increasing rate, then constant	Fairly constant, slightly increasing, then falling abruptly and rapidly
Precipitation	None, then gradual steady rain, then rapidly falling off to none	None, then heavy brief shower, perhaps thundershower, clearing rapidly

*From Air Mass Analysis. Jerome Namias and others. American Meteorological Society, 1936.

PREPARATION OF HORSE DANDER MATERIAL

Horse combings were obtained from a riding academy. Primary separation of hair and fat from the dander was accomplished by repeated decantations with ether on filter paper. The material was then washed with several baths of ether until there was no appreciable color in the ether washings and was then extracted with a Soxhlet apparatus for three hours. After drying in the air, it was sifted in a 100 mesh screen. In addition to Ratner's technique, we ground this dry, fat-free dander in a ball mill for eighteen hours. At this time the particles of dander, when blown on a vaselined glass slide, varied from 0.1 to 10 microns in size.

After the initial experiments a few of the animals developed eye infections from scratching. This difficulty was overcome by dropping 10 per cent argyrol into the eyes of all pigs after each exposure. That contamination of the horse dander with unusual bacteria or fungus was the cause we disproved by puffing it over Petri dishes containing blood agar and Sabouraud's culture media. A comparison of these plates with those placed in a clean chamber over which filtered air was blown showed that the bacteriologic growths which developed were identical in number and kind. Both plates contained the common air-borne types of bacteria.

The horse dander extract was prepared simply by soaking the fat-free dander in Evan's extracting saline solution for ten hours. It was then filtered through No. 271 Reve Angel paper pulp, centrifuged, re-

filtered, passed through a Seitz filter, and tested for sterility. Further filtering or adjustments of pH were not necessary because control pigs injected intravenously with this solution showed no clinical symptoms and no significant histopathologic changes in the respiratory mucous membranes or lungs.

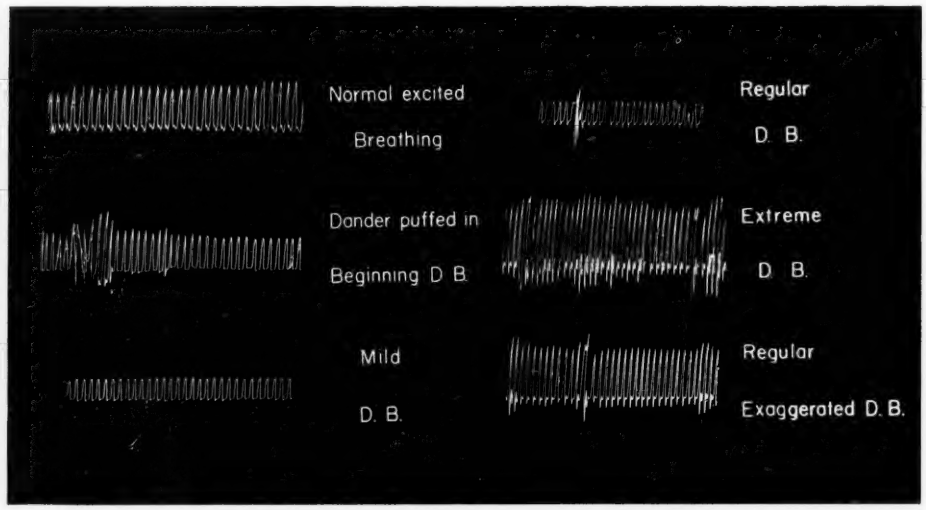

Fig. 2.—Kymographic records of expiratory dyspnea. *D.B.*, Expiratory dyspnea.

TYPES OF BREATHING OBSERVED IN SENSITIZED ANIMALS

Fig. 2 shows "anaphylactic breathing" in the guinea pig. The tracings record the variations in air displacement in a body plethysmograph when a guinea pig experiences the dyspnea and expiratory difficulty from inhalant anaphylaxis in the experimental chamber under dry, hot atmospheric conditions. For the physical system used, the normal, rapid, and excited breathing all show a small dicrotic notch at the end of the expiratory impulse. When the horse dander is blown in upon a sensitized pig and the animal reacts, the dicrotic notch moves into the ascending arm of the inspiratory phase. This vibration also becomes more visible as the expiratory difficulty increases. Furthermore, the lowest point of the expiratory phase was found to be below the base line. Such a graphic picture of the breathing seen in typical experimental anaphylactic shock stresses the importance of clinical observations of the different forms of dyspnea. It acts as a clinical guide in determining the presence or absence of sensitization in the animal. This respiratory distress is not unlike acute forms of asthma seen in man.

DISCUSSION OF ILLUSTRATIVE CHARTS

The first experiments were planned for the purpose of becoming acquainted with the apparatus described, and with the technique of

sensitization and shock under controlled weather conditions. A discussion of three representative charts taken from a large series of experiments will clarify the value of this method of study and the fundamental principles underlying its use.

Certain constants were established. Ten puffs of dander, representing 0.1 Gm., were blown into the chamber every five minutes. The circulation of filtered air was placed at 0.15 cubic feet per minute. Barometric pressure in the warm compartment was 3 inches of water below the room air, while that in the cold chamber was regulated to 3 inches above that of room air. These latter are, of course, relatively negligible quantities but are pointed in the right directions for warm and cold fronts. It should be noted again that the animals were subjected to a "cold front" when taken from room air and placed in the cold compartment and a "warm front" when placed in the hot chamber. More extreme changes occurred when the pigs were shifted, for example, from the hot, dry to the cold, moist chamber. The water nebulizer in the cold compartment was adjusted to give droplets of moisture 0.6 to 45 microns in size to simulate fog.[10]

Drinker has emphasized the many meteorologic factors which might affect particles of the size used in our experiments. Particles this size are very active physically, chemically, and electrically, and the reactions depend not only upon the nature of the substance but upon the temperature, barometric pressure, humidity, and air movement in the atmosphere. For example, it is probable that a rising temperature decreases the adsorption of a gas film on particles less than 40 microns in size and that a rising barometric pressure increases the gas film adsorption. Wetting of dust particles will not take place unless the spreading force of the liquid is great enough to drive off the gas film.

Ratner states that a liquid antigen is more likely to produce a general sensitization than a dry one, the dry one causing only a local sensitization in a number of instances. He found that only a percentage of the uteri of guinea pigs sensitized by the inhalation of dry dander reacted by the Dale-Shultz technique, whereas Kallos and Pagel found that all uteri responded in animals sensitized by a sprayed liquid allergen. It may be that the dry dander used by us is more easily moistened under certain meteorologic conditions and therefore more readily able to penetrate the respiratory membranes.

Figs. 3, 4, and 5 show the day-to-day relationships of outdoor weather, room air, and experimental chamber weather to the exposure and reactions of the guinea pigs. Rappaport, Welker, and Nelson[11] reported that asthmatic patients who were sensitive to pollen and had improved in an air-conditioned room (filtered air, control of humidity and temperature) had trouble while still in the room when there was a great outside meteorologic change, such as the passage of a cold front. Petersen[12]

stressed the relationship of asthmatic attacks to weather change. It is important therefore to study the outside weather in order to interpret properly the data on experimental weather and its effect on inhalation sensitization and shock.

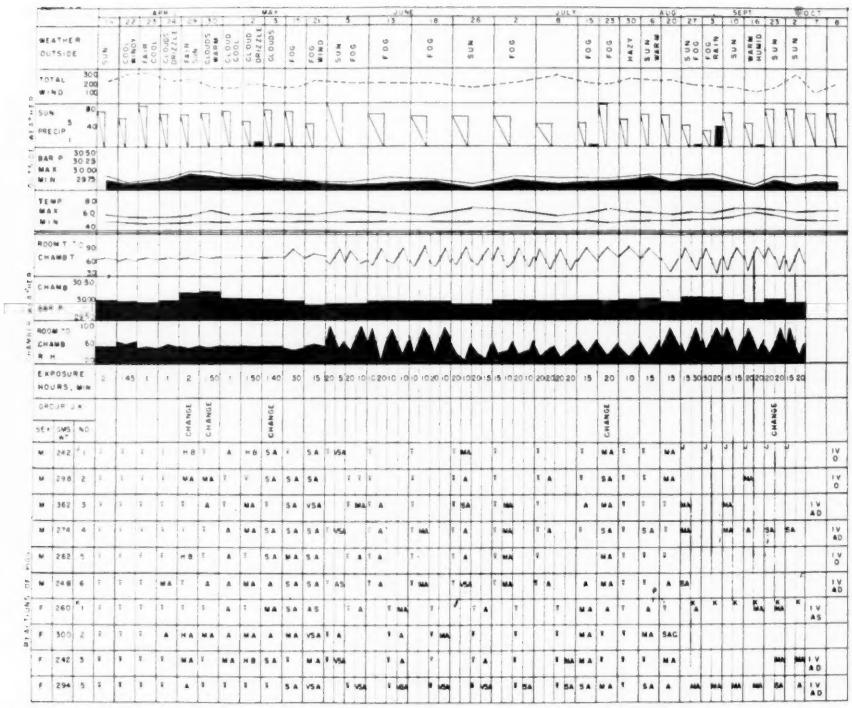

Fig. 3.—Record of experimental dyspnea. *A*, expiratory dyspnea (experimental respiratory anaphylaxis); *AD*, anaphylactic death; *AS*, anaphylactic shock; *HB*, heavy breathing; *MA*, mild expiratory dyspnea; *SA*, severe expiratory dyspnea; *VMA*, very mild expiratory dyspnea; *VSA*, very severe expiratory dyspnea; *IV*, intravenous injection; ⊔, exposed.

Fig. 3 summarizes the observations on ten guinea pigs sensitized in the chamber under air conditions comparable to that of room air (68° F., and 50 to 55 per cent R.H.). They were exposed to the horse dander over a period of five days for a total of five hours and forty-five minutes before any changes in breathing occurred. After ten hours' exposure in the chamber most of the pigs showed very definite dyspnea characteristic of the type described above. These sensitized pigs were shocked under various experimental weather conditions during a period of 5.5 months, and as a final check they were then all injected intrajugularly with 0.5 c.c. of horse dander extract. Five died in typical anaphylactic shock. Three had no symptoms, and one had severe shock but recovered. In general, the reactions followed a pattern similar to that described by Ratner. There were variations in degree of reaction in the same animal on different exposures; some lost their sensitivity

as demonstrated by the negative reaction to the final intravenous test; some reacted quite consistently at each exposure while others did not; antianaphylaxis was not so apparent as in animals sensitized by parenteral injection. In Ratner's more recent work he found that the optimum exposure period necessary for inhalation sensitization under room conditions was seven hours, that is, one hour a day over a period of seven days. Our findings approximate his results in this respect.

To describe weather analysis, June 13, 1940, is taken as an example (Fig 3). It is seen that the sensitized animals were taken from room air at 60° F., 55 per cent R.H., and placed in the cold compartment at 43° F., 100 per cent R.H. They were conditioned here for ten minutes then shifted to the hot, dry chamber at 90° F., 30 per cent R.H., and the dander was blown in. Eight of the ten pigs had definite respiratory anaphylaxis. It may be noted from the chart that the weather outside was foggy but with a total of 65 per cent sunshine; total wind was 200. Comparing the temperature and barometric pressure of June 13 with that of several days before and after, as found in the San Francisco Monthly Meteorological Summary for June, we found that on June 13 the barometric pressure described a slight fall but both the maximum and minimum temperatures remained almost constant. Although regular diurnal changes are common in San Francisco, barometric and weather changes of gross caliber are infrequent. For this reason a trained meteorologist suggested that we study the isobars and wind directions on the daily San Francisco Weather Maps. It is apparent that the air mass movement coming into San Francisco from the ocean can be classed as "polar Pacific," "moderate polar Pacific," and "tropical Pacific." For example, on April 30, 1940, the direction of air changed from "polar" to "tropic." We noted this on the chart by the word "change." On June 13 there was no such change.

Meteorologists are interested in the lesser known weather variables, and physiologists and physicists are studying the possible effects on animal life. Loeb[13] dismisses the possibility that ions in the air have any physiological significance. He states that, even if particles totaling 1 c.c. of dust were inhaled in 100 seconds and all particles charged with one sign, this would equal only 2×10^{-8} amperes. This current density spread over the large area of the nasal and respiratory mucous membrane could have no physiologic effect. He believes that one should investigate the effects of dust, ozone, N_2O_4, SO_2, NH_3, CO_2, CO, and water vapor. This conclusion is significant, but still one wonders if the allergenicity of the air-borne dust may not be affected by some electrical force.

Therefore our final conclusion for June 13, in regard at least to known weather variables, is that any possible weather influence on guinea pig reactions came from the experimental weather rather than

the outside meteorologic factors. For our study of inhalant sensitization, outside weather, as analyzed above, was taken into consideration for each exposure day.

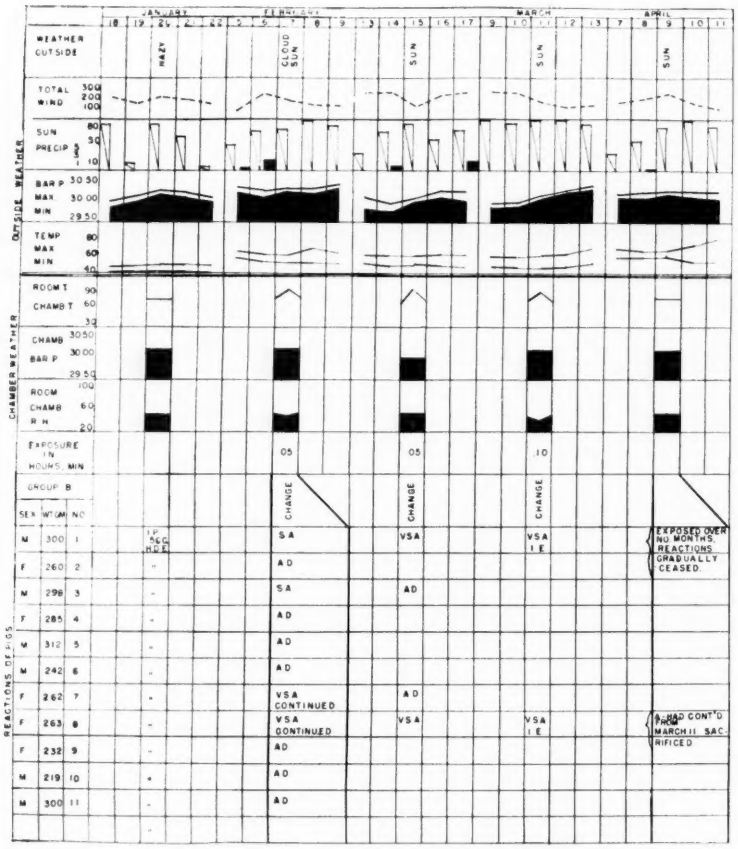

Fig. 4.—Record of experimental dyspnea.

Fig. 4 illustrates the use of the air-conditioned chamber in shocking animals sensitized by intraperitoneal injection. On Jan. 20, 1940, eleven pigs were injected with 0.5 c.c. horse dander extract. Eighteen days later they were placed in the chamber at 90° F., 40 per cent R.H., and 0.1 Gm. of dry dander was blown in. In two minutes, seven of the pigs died in typical anaphylactic shock, as proved by symptoms and histologic sections. One week later two more died in anaphylactic shock following exposure. Of the remaining two, one decreased in sensitivity over a period of months until completely refractory and one developed a chronic respiratory anaphylaxis. At autopsy lung sections showed areas of ballooning of alveoli as found in acute anaphylaxis and also large areas of atelectasis. There were no signs of pneumonia. When the results of this group are compared with those

of a similar group reported by Ratner, it seems not unlikely that the dry, hot air environment and grinding of the dander to a smaller particulate size were important in our method, for nine of eleven of our animals died; of Ratner's thirty-nine pigs, only five died in anaphylactic shock on exposure to dry horse dander in room air.

According to Opie,[8] an allergen is held in a specifically sensitized tissue membrane but passes through a normal one. When the reactions of the animals sensitized parenterally (Fig. 4), are compared with those sensitized by inhalation (Fig. 3), it may be concluded that the former are sensitized generally but possibly retain a more permeable respiratory membrane than the latter. Thus, they are more apt to die in anaphylactic shock because the allergen penetrates the respiratory membrane and probably comes in contact with more shock tissue throughout the lung and elsewhere. Furthermore, those sensitized by inhalation of dry dander retain more of the allergen locally, the local tissue being more highly sensitized by direct contact with the allergen.

Because of a reasonable doubt that normal unsensitized guinea pigs may have abnormal breathing on exposure to dry horse dander or may react in some toxic manner to the intravenous injection of horse dander extract, we exposed six normal pigs of 250 Gm. each to the dry horse dander in hot, dry air simultaneously with several sensitized animals. No clinical reactions occurred in the normal pigs. When they were injected intravenously with 0.5 c.c. of horse dander extract on the same day that the sensitized animals were injected, no reactions took place and no changes were observed grossly or microscopically.

The guinea pigs represented in Fig. 5 show most of the symptoms which may be anticipated when animals are sensitized by inhalation and subjected to changing environmental conditions before exposure to the allergen. Sensitization in them was accomplished in air somewhat warmer and drier than room air. On September 19 they were shifted from the hot, dry compartment at 92° F., 35 per cent R.H., to the cold, moist, chamber at 38° F., 100 per cent R.H., three times, remaining in each temperature change twenty minutes. They were then shifted again to the hot, dry chamber and the dander was blown in. Two had anaphylactic shock and undoubtedly would have died if they had not immediately been removed to room air. One had mild dyspnea, and two had very definite respiratory anaphylaxis. As noted in Fig. 5, they were exposed to this type of changing weather every week for four more weeks. Reactions occurred on their first exposure to the dander in cold, moist air but after this, on October 8 and 28, November 18, and December 3, no reactions took place in cold, moist air although the animals continued to react in the hot, dry air. It is interesting that so many different responses occurred in this small group of animals.

Nos. 1 and 6 died in anaphylactic shock on intrajugular injection of horse dander extract 4.5 months after their first sensitizing exposure. They had reacted consistently to the dander on exposure in hot, dry air. No. 4 became gradually refractory over the period of September 19 to 30 and did not react to the intravenous injection. No. 3 developed a pneumonic infection discovered at autopsy, while No. 2 developed chronic respiratory anaphylaxis. Clinically No. 2 did not have the same type of discomfort as No. 3. Audible moist râles were heard in pig No. 3, but No. 2 had only an expiratory dyspnea. Both pigs lost weight, but No. 3 looked much more toxic.

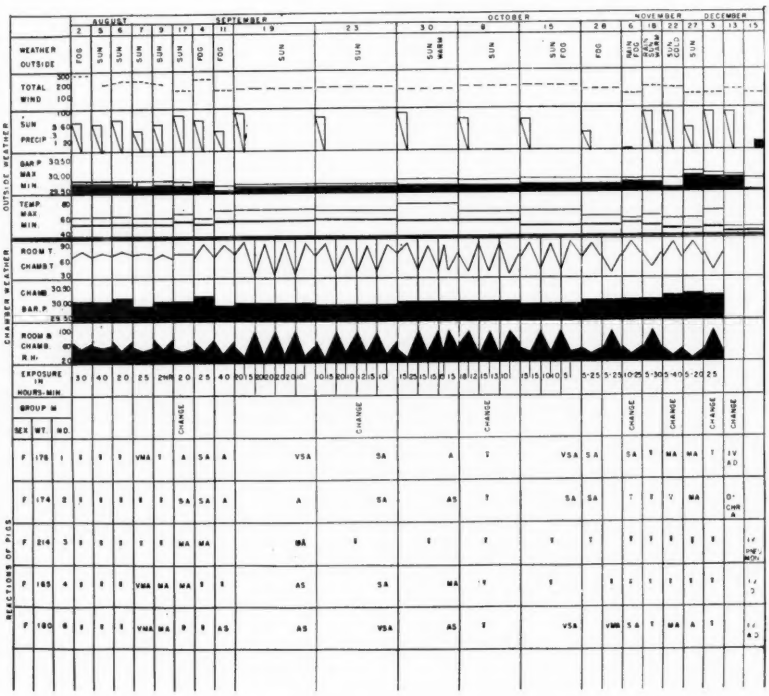

Fig. 5.—Record of experimental dyspnea.

DISCUSSION

When animals are subjected to changes in environment, a number of factors must be considered. Mudd and Grant[14] found that, when body surfaces are cooled, body temperature remaining the same, the temperature of the nasal membrane falls due to vasoconstriction. This change is variable in different subjects. Spiesman and Arnold[15] demonstrated that, if body temperature itself falls, the nasal membrane temperature rises, and conversely, if body temperature rises, membrane temperature falls. However, in what he calls the hypersensitive group, the opposite occurs. Changes in energy requirements and a loss and redistribution

of metabolites no doubt also occur.[16-18] In pig No. 3 (Fig. 5), we have an example of respiratory infection taking place in an animal exposed to a combination of particulate matter and changes of weather. Most writers (Kerr and Lagen,[19] Yaglou,[20] Mudd, Goldman, and Grant,[21] Brundage and Bloomfield[22]) believe that factors of this kind are important in the incidence of infection. Chodousky,[23] however, carried out many experiments but could discover no changed susceptibility to infection when animals were chilled. Petersen[12] summarized his ideas by stating that, when the animal is exposed to "quick cold," there is a "retreat" into the body—the skin vessels contract and the blood pressure increases; that is, vascularization of the interior is greater, and the body cells are less permeable. In the hot climate the peripheral blood vessels dilate; the blood pressure is lowered; vascularization of the interior is less; and the basal metabolic rate is depressed. If the temperature is very high, there are an alkalosis and a greater irritability of the autonomic nervous system.

It is apparent, therefore, that in the interpretation of results many factors must be considered when the method of inhalant sensitization is employed under controlled and varied meteorologic conditions. Such an approach to the problem may enable us to determine many of the important variables.

SUMMARY

1. An air-conditioned experimental chamber is described for the study of inhalant sensitization in laboratory animals.

2. A kymograph record is shown of the breathing found in sensitized guinea pigs suffering from sublethal respiratory anaphylaxis. This suggests a differential diagnosis of different forms of dyspnea observed.

3. Inhalant sensitization was produced under controlled meteorologic conditions, and this follows a pattern described by others.

4. The method of using the air-conditioned chamber and some of the fundamental physical and physiologic principles underlying its use are presented.

5. The illustrative charts presented give briefly the preliminary results of a two-year study. The part played by experimental weather in inhalant sensitization and its role in modifying the susceptibility of the respiratory mucous membranes to sensitization will be presented in subsequent papers.

We wish to acknowledge the valuable help of Allan B. Conant and Keith O. Alexander, engineers, in the construction of the apparatus, and to thank Montgomery Brothers, Ventilating Equipment Co., and Cyclops Refrigerating Co. for gifts and loan of materials. We are indebted also to Mr. Irving White for his technical aid.

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PROTEINS OF RAGWEED POLLEN

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AN ATTEMPT to determine the fraction of the ragweed pollen containing the active principle responsible for the symptoms of hypersensitivity suggested to early investigators the need for a study of the chemical composition of pollen with special consideration of the nitrogen distribution.[1]

One of the earliest experimenters in the fractionization of the ragweed pollen was Heyl. He determined the percentage composition of the pollen and ascribed a quarter part to protein.[2]

Koessler, on the basis of her analysis of ragweed pollen, concluded that it was composed of 21.1 per cent inorganic and 78.9 per cent organic matter. The inorganic portion consisted of moisture and ash. She described the organic portion as being reducing sugars after hydrolysis, ether-soluble lipoids, a portion insoluble in ether but soluble in 95 per cent alcohol, extractives, and an insoluble residue. She further determined the distribution of nitrogen in each of the organic fractions. At that time, however, no accurate method had been developed to differentiate quantitatively the protein from the nonprotein nitrogen.[1]

Later Caulfeild, Cohen, and Eadie[3] prepared three fractions of pollen by chemical methods. They labeled them as alcohol extract, albumin-proteose and the proteose-glutenin fractions. They demonstrated with ease and regularity that guinea pigs could be sensitized by both the albumin-proteose and the proteose fractions of the ragweed. By clinical tests, using the scratch method, they demonstrated that each case would show an individual selective specificity to the different fractions and that positive results occasionally would be obtained when the use of whole pollen would give negative results.

More recently, Johnson and Rappaport[4] showed in their experiments that the giant ragweed pollen contains at least two antigens. They believed one to be globulin and the other albumin. They concluded that the active principle of ragweed pollen is of protein nature or that it clings to a protein moiety; that under proper conditions this principle is responsible for the production of precipitins in the injected rabbit; and finally, that the nonprotein fractions of the pollen are clinically inert.

Hecht, Rappaport and Welker[5] showed that the protein of ragweed pollen could be separated into proteins of large molecular size which

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were responsible for the precipitin reaction and proteins of small molecular size which were responsible for the skin reactivity. This observation was noted previously on grass pollens by Benjamins and his collaborators.[6]

Harley concluded that the skin reactivity was associated with the large aggregate.[7]

Hecht, Rappaport, and Welker[5] studied the immunizing properties of the small fraction and found that it did not produce a hyposensitization in ragweed-sensitive patients. They further indicated that the immunizing powers were increased when the small fraction was used with the large molecule. This confirmed the observations made by Benjamins and his collaborators[6] on grass pollens.

While the previous workers have been able to prepare the small molecule free from the precipitin and anaphylactic-reacting protein, none succeeded in freeing the large protein molecule from the skin-reacting small molecule. Hence, the question of the skin reactivity of the large protein molecule remained unsettled.

EXPERIMENTAL DATA

The plan of the work was to take successive extracts of a definite quantity of pollen with the same volume of solvent and determine the nitrogen concentration, the skin reactivity, and the precipitin response in each extract.

It had been shown previously that under such conditions the decline in the nitrogen concentration in Extracts 1 to 10 would produce a curve that was in most respects similar to the decrease in skin reactivity. It had not, however, been demonstrated what would happen to the precipitin response under similar conditions.

One hundred cubic centimeters of distilled water were added to 10 Gm. of ether-extracted pollen. The pollen was allowed to extract for twenty-four hours with occasional shaking, and then the solution was centrifuged. The supernatant fluid was drawn off. Another 100 c.c. of water were added to the pollen, and the process was repeated. This was done until ten extracts had been obtained and was carried out without removing the pollen from the centrifuge bottle at any time.

On each of these extracts determinations were made of the nitrogen concentration, the skin reactivity, and the precipitin response. The nitrogen concentration was established by the micro-Kjeldahl method. It was found that in the first four extracts there was a rapid fall in the nitrogen content, but after that the decline was extremely gradual, maintaining itself at almost a fixed level. The values gave the curve as seen in Fig. 1.

To determine the skin reactivity, scratch tests were done on twelve dispensary patients of proved sensitiveness to ragweed pollen. Four

groups were designated by the varying degrees of pollen sensitivity; Group A was the most sensitive, and Group D, the least sensitive. Three patients were tested in each group, and the results were recorded as ranging from one plus to four plus. A 3 by 3 mm. wheal was indicative of a one-plus reaction; a 5 by 5 mm. wheal, a two-plus reaction; a 7 by 7 mm. wheal, a three-plus reaction; and a 10 by 10 mm., a four-plus reaction. The results are given in Table I.

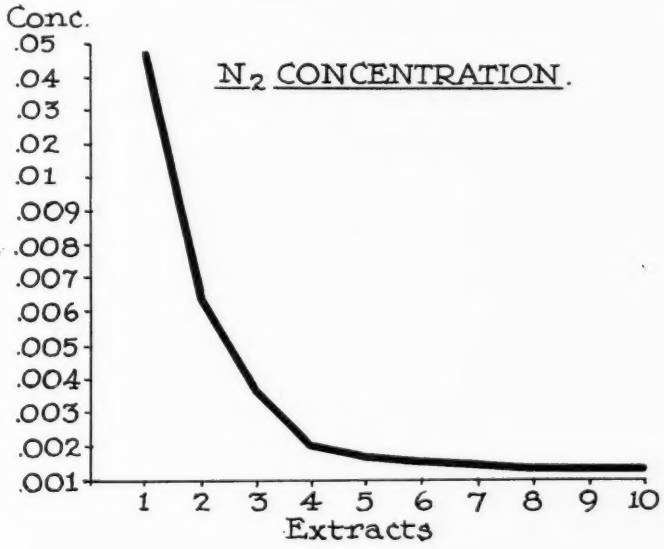

Fig. 1.

TABLE I

EXTRACT	GROUP A	GROUP B	GROUP C	GROUP D
1	++++	+++±	++	+
2	+++	+++	+	±
3	++±	++	±	±
4	+±	+	±	–
5	+	±	±	–
6	±	±	–	–
7	±	±	–	–
8	–	–	–	–
9	–	–	–	–
10	–	–	–	–

When the data are plotted (Fig. 2), the curve is similar to that seen in the nitrogen concentration. This is true in all four groups. However, the skin reactivity, in even the most sensitive patients, had completely disappeared by the eighth extraction.

Finally, the precipitin response was determined with pollen antiserum. The antiserum was prepared by the aluminum cream method of Hektoen and Welker.[8] The results are seen in Fig. 3. Here, unlike the first two curves that more or less parallel each other, there is no such cor-

relation. The response fell off more slowly and never attained a very low level, i.e., even in the tenth extract there was a positive reaction in a dilution of 1:10,000 (Fig. 4).

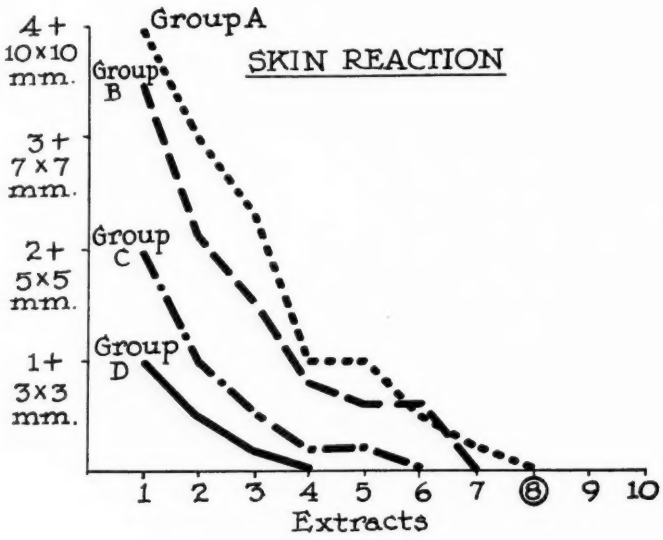

Fig. 2.

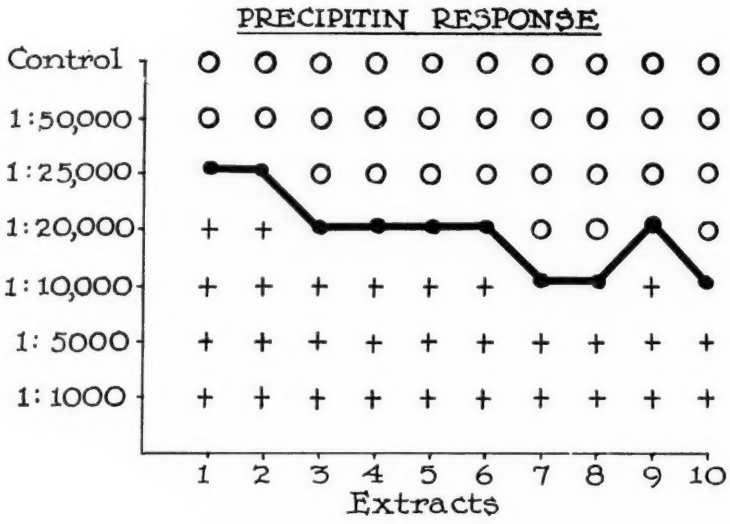

Fig. 3.

A possible explanation of these results is the difference in solubility of the large and small protein molecules of pollen. The small molecule, not in combination with the large, is very soluble. The rapid decrease in nitrogen concentration in Extracts 1 to 4 is due for the most part to the rapid extraction of the small molecule and is only slightly dependent upon the small amount of the large molecule in the solution. The

fact that most of the readily soluble portions are taken out early also accounts for the leveling out of the nitrogen curve in the later extractions. Finally, the maintenance of the curve is due to the relatively insoluble large molecule.

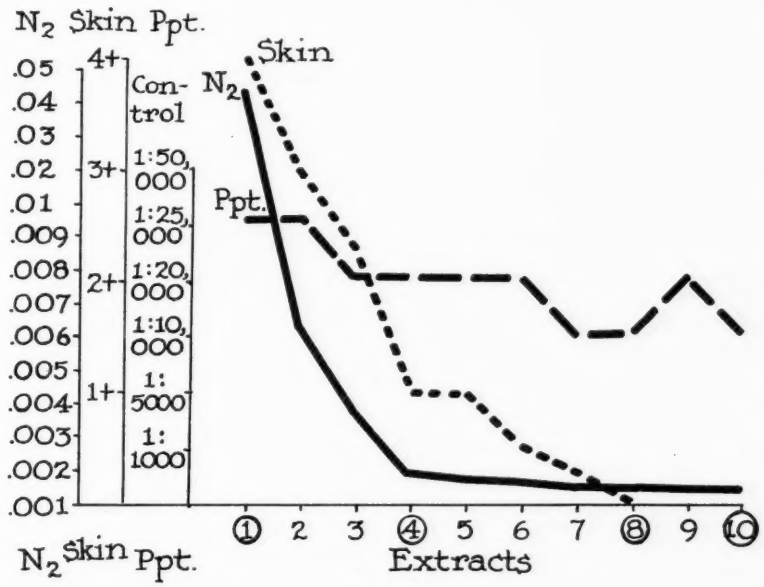

Fig. 4.

The skin response is likewise explained on a similar basis, i.e., the rapid withdrawal of the small molecule which accounts for the completely negative response after the eighth extraction.

With the same reasoning, the precipitin response, probably due to the large molecular aggregate, would be expected to maintain itself with only slight decline from extracts 1 to 10. This is borne out experimentally, and it is noteworthy that at the tenth extraction there is still a positive reaction in 1:10,000 dilution.

The experimental results show a method for the quantitative separation of the small protein of pollen from the large. Since the small molecule is responsible for the skin reactivity and this response had completely disappeared by the eighth extract in even the most sensitive patients, it is reasonable to assume that all appreciable amounts of the skin-reactive protein had been removed.

Since the precipitin reaction is dependent upon the large protein molecule and it maintained itself to a marked degree, it is likewise reasonable to assume that the large molecule is still present. This is supported by the fact that the nitrogen concentration is maintained at almost a fixed level after the skin reaction is absent.

The experiment was repeated, using twenty extractions. All of these extracts beyond the one which showed complete disappearance of the

skin reactivity (Extract 9 in this series) were concentrated by evaporation. In the concentrated solution, nitrogen concentration, skin reactivity, precipitin response, and anaphylactic reaction were determined. The results were as follows: The nitrogen concentration was 0.009 mg.; the skin reactions were negative; the precipitin response was positive in a 1:20,000 solution; finally, anaphylactic reactions were demonstrable. In two of the guinea pigs mild reactions, such as tufting of the hair on the neck, micturition, defecation, nose itching, and twitchings, were present. The reactions lasted about twenty minutes and slowly disappeared. One guinea pig died after going through typical shock. The control was unaffected. This indicates a maintenance of nitrogen concentration, absence of skin reactivity, maintenance of precipitin response, and anaphylaxis.

CONCLUSIONS

1. There appear to be two protein fractions in ragweed pollen. One is of large molecular size and is responsible for the precipitin reaction with pollen rabbit antiserum and for anaphylaxis in the sensitized guinea pig. The other fraction is made up of small protein molecules and is responsible for the skin reactivity.

2. After twenty consecutive extractions, there were maintenance of nitrogen concentration, positive precipitin response, and experimental anaphylaxis. The skin reactivity had long since disappeared.

3. When a large protein molecule of pollen had been quantitatively freed from the small molecule, it failed to show any skin reactivity. Hence the skin reactivity appears to be a property of the small molecule only.

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EVIDENCE OF THE MULTIPLICITY OF ALLERGENS AND REAGINS IN COTTONSEED SENSITIVENESS

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ISOLATION as well as chemical and immunologic properties of a polysaccharide-protein fraction (CS-1 or CS-1A) from cottonseed have been described in previous publications.[1-7] The unusual stability of the active components of this fraction permitted employment of procedures drastic enough to effect a high degree of purification. The scheme evolved for the isolation of CS-1 was based on selection of procedures which yielded products demonstrably more skin reactive than preceding fractions. This scheme, however, might yield only the most potent or most stable of several allergenic components present in cottonseed. The object of the present study, therefore, was to determine whether the cottonseed allergen present in CS-1, or in the more highly purified picrate derivative CS-5, represented the sole allergen or only one of several water-soluble allergens of the cottonseed embryo.

Coca and Grove[8] demonstrated that sites passively sensitized with serum from a patient sensitive to both cottonseed and kapok did not react to kapok seed extract after desensitization with cottonseed extract. If, however, the sites were desensitized first with kapok seed extract, they were still reactive to the cottonseed extract. These results indicated the existence of a common allergen in the seeds of the botanically related cotton and kapok and, moreover, that cottonseed contained at least two allergens. (Sherman and Stull[9] have demonstrated, however, that with certain sera unrelated antigens sometimes effect cross neutralization of reagins.) The same principle was applied in the present investigation to determine whether the allergen concentrated and purified in the fraction CS-5 represented one component distinguishable from other different allergenic components of unfractionated extract of cottonseed. It was assumed that reagins specific for the allergen contained in CS-5 could be exhausted if a reaction were induced in a passively sensitized site by a relatively large dose of this fraction. If the first dose of CS-5 exhausted the reagins specific for its allergen, no response would be induced by administration of a second large dose of the same fraction after the first reaction had subsided. Thereafter, a positive reaction in a site desensitized with fraction CS-5, if induced

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by administration of unfractionated water extract of cottonseed, would demonstrate the presence of multiple allergens in cottonseed and, axiomatically, would confirm the existence of multiple reagins for cottonseed in the serum employed for passive sensitization.

Blood sera from seven cottonseed-sensitive patients were used in this study. Preliminary tests were performed in the usual manner by injecting the allergen intracutaneously into the sensitized site and making a control injection elsewhere on the arm.[10] However, the results presented in this report were obtained by adaptation of the technique described by Lippard and Schmidt.[11] The indirect administration of the test solutions obviated comparisons with control injections and avoided the possibility of erythema from nonspecific irritation, particularly with unfractionated cottonseed. Positive reactions in the sensitized sites subsided with no visible trace of irritation. Subsequent positive and negative reactions were thus recognized and evaluated solely by examination of the passively sensitized sites.

Recipients were selected at the Allergy Clinic of Providence Hospital, Washington, D. C. They exhibited no evidence of cottonseed sensitiveness by history or tests.

The fractions used for this study are described in detail in previous papers. A brief description of the material used follows.

CS-5.[2]—The active picric acid derivative of CS-1 was adsorbed on activated aluminum oxide and eluted. The resulting protein picrate was designated CS-5. A 1:1,000 solution of CS-5 was prepared by dissolving 100 mg. of the picrate in 25 ml. of 0.01 N sodium hydroxide. The alkaline solution was diluted to 80 ml., and the pH was adjusted to 7.4 with 0.05 N hydrochloric acid. This solution was diluted to 100 ml., and 0.9 Gm. of sodium chloride was added. The solution was sterilized by Seitz filtration.

CS-13A.[2]—The picric acid-free protein recovered from the picrate CS-5 was designated CS-13A. CS-13A contained 20.1 per cent total nitrogen (ash-water free basis). A 1:1,000 stock solution of CS-13A was prepared by dissolving 100 mg. of the protein in 100 ml. of physiologic salt solution. Solutions were sterilized by Seitz filtration or by heating to 100° C.

Aqueous Extract of Cottonseed (1:10).—Twenty grams of benzene-extracted cottonseed were extracted by gentle shaking with 200 ml. of distilled water. The insoluble cottonseed residue was separated by centrifuging. Sodium chloride was added to the extract to physiologic concentration. The solution was then sterilized by Seitz filtration.

TECHNIQUE OF TESTING

Single sites were uniformly sensitized on the right upper arm of recipients by using 0.1 ml. of diluted or 0.05 ml. of undiluted serum

from cottonseed-sensitive patients. After an interval of at least seventy-two hours 0.5 ml. of a 1:1,000 solution of CS-5 was injected intramuscularly into the left arm of the recipient. Itching of the sensitized site was reported by the patients within a few minutes after the injection, and positive reactions developed in twenty to sixty minutes. After an interval of not less than seventy-two hours the sensitized sites were tested for exhaustion of reagins specific for CS-5 by a second intramuscular injection of 0.5 ml. of 1:1,000 CS-5 into the left arm of the recipient. In no instance was there a positive reaction at the sensitized site following this second test, thus indicating complete desensitization of reagins for CS-5 by the initial dose. After another interval of a minimum of seventy-two hours a dose of unfractionated water extract was injected intramuscularly into the left arm. Positive reactions which developed in the sensitized sites following this injection of unfractionated aqueous extract indicated the presence of more than one allergen in cottonseed.

The results of a series of passive transfer tests with serum from seven cottonseed sensitive patients are shown in Table I.

A massive dose of CS-5 was used in desensitizing the sites in order to demonstrate the degree of separation of this allergen from other allergens of the cottonseed. It was of interest, therefore, to determine the minimum quantity of allergen which would desensitize a site to a subsequent tenfold quantity of the same substance by the technique employed. For this purpose fraction CS-13A, which represents the picric acid-free, active component of CS-5, was used. The results in Table II demonstrate that a dose of 10 micrograms of CS-13A was required to desensitize completely sites sensitized with 0.05 ml. of one of the undiluted serums. Subsequent injection of 100 micrograms of CS-13A produced no further reaction in sites desensitized with 10 micrograms.

Neutralization of reagins in serum by allergens provides a sensitive method for determining specificity. The authors are indebted to Dr. Arthur Stull and Dr. Wilson M. Wing for the results of a series of tests (conducted at Roosevelt Hospital, New York) involving this technique.[12, 13] The method employed consisted of mixing equal parts of serum with physiologic salt solution containing varying quantities of fraction CS-13A or unfractionated cottonseed extract. Duplicate sites were sensitized with 0.1 ml. of these mixtures. Forty-eight hours after sensitization the sites were tested with CS-13A or unfractionated cottonseed extract. Results of a series of these tests with serum from six cottonseed-sensitive patients are shown in Table III. These results show that CS-13A desensitized only part of the reagins in sera (Sher, Hough, M. J., and L. C.). Unfractionated cottonseed produced further reaction in these sites. However, CS-13A completely desensitized the reagins in sera Stein and Q. N. to further test with cottonseed extract.

TABLE I

RESULTS OF PASSIVE TRANSFER EXPERIMENTS WHICH DEMONSTRATE THE MULTIPLICITY OF ALLERGENS IN COTTONSEED

COTTONSEED SENSITIVE SERUM			RECIPIENT†	ALLERGENIC SOLUTION‡			
				FIRST INJECTION OF 500 MICROGRAMS OF CS-5	SECOND INJECTION OF 500 MICROGRAMS OF CS-5	THIRD INJECTION WITH 0.5 ML. OF UNFRACTIONATED AQUEOUS COTTONSEED EXTRACT	
SERUM	QUANTITY (ML.)	DILUTION*		REACTION§	REACTION§	REACTION§	DILUTION
L. C.	0.1	1:1	T. W.	4+	0	±	1:10
	0.1	1:1	R. G.	4+	0	0	1:10
	0.1	1:1	W. P.	4+	0	0	1:10
	0.1	1:1	B. D.	4+	0	0	1:10
L. B.	0.1	1:10	A. Ca.	4+	0	3+	1:10
	0.1	1:10	A. Cu.	4+	0	2+	1:10
	0.1	1:10	C. W.	4+	0	1+	1:10
	0.1	1:10	J. L.	4+	0	2+	1:10
	0.1	1:10	L. S.	4+	0	1+	1:10
	0.1	1:10	C. W.	4+	0	3+	1:10
	0.1	1:10	J. W.	4+	0	0	1:10
	0.05	Undil.	W. E.	4+	0	2+	1:50
	0.05	Undil.	H. R.	4+	0	4+	1:100
C. A.	0.1	1:10	G. C.	3+	0	±	1:10
	0.1	1:10	R. J.	4+	0	1+	1:10
	0.1	1:10	M. S.	3+	0	0	1:10
	0.1	1:10	E. K.	4+	0	2+	1:10
	0.1	1:10	B. S.	4+	0	2+	1:10
Q. N.	0.1	1:1	M. D.	0	0	0	1:10
	0.1	1:1	I. J.	0	0	0	1:10
	0.1	1:1	C. R.	0	0	0	1:10
	0.1	1:1	I. M.	2+**	0	0	1:10
	0.1	1:1	H. G.	2+**	0	–	1:10
B. H.***	0.05	Undil.	T. S.	4+	0	0	1:100
	0.05	Undil.	A. B.	4+	0	0	1:100
	0.05	Undil.	V. G.	4+	0	0	1:100
I. J.	0.05	Undil.	M. D.	4+	0	0	1:10
	0.05	Undil.	C. H.	4+	0	3+	1:10
	0.05	Undil.	B. T.	4+	0	0	1:10
	0.05	Undil.	M. R.	4+	0	±	1:10
L. J.	0.05	Undil.	B. P.	4+	0	3+	1:10
	0.05	Undil.	W. C.	4+	0	3+	1:10
	0.05	Undil.	M. C.	4+	0	4+	1:10
	0.05	Undil.	B. W.	4+	0	3+	1:10
	0.05	Undil.	M. S.	4+	0	3+	1:10
	0.05	Undil.	M. R.	4+	0	3+	1:10

*Serum was diluted with sterile physiologic salt solution.

†Recipients were uniformly sensitized on the right upper arm with the indicated amount of serum from cottonseed-sensitive patients.

‡Allergenic solution was injected intramuscularly into the left arm. The first dose of CS-5 was given at least 72 hours after sensitizing the site. A minimum of 72 hours then elapsed between subsequent injections. During this time wheals produced by the previous injection completely disappeared.

§Wheals developed within 15 to 45 minutes. A 4+ reaction indicates a wheal of at least 15 mm. diameter. Many wheals were 25 to 40 mm. in diameter. A 3+, 2+, or 1+ reaction indicates a wheal ¾, ½, or ¼ respectively, of the size of the original reaction.

**Wheals 4 to 7 mm. in diameter.

***This serum was obtained from Dr. Mathew Walzer of the Jewish Hospital of Brooklyn.

These results are in agreement with those obtained by the in vivo desensitization method used with CS-5.

TABLE II

PASSIVE TRANSFER EXPERIMENTS SHOWING THRESHOLD QUANTITY OF COTTONSEED ALLERGEN (CS-13A) REQUIRED TO DESENSITIZE SITES

RECIPIENT*	INJECTION NO.†	SOLUTION OF CS-13A INJECTED IN LEFT ARM	
		QUANTITY‡ (MICROGRAMS)	REACTIONS§ (MM.)
A. A.	1-4	10	18
	2-3	100	0
B. D.	1-4	10	35
	2-17	100	0
L. E.	1-4	1	0
	2-3	100	20
	3-4	100	0
I. J.	1-4	1	0
	2-3	100	13
	3-4	100	0
L. S.	1-4	0.1	6
	2-3	10	10
	3-4	100	0
F. W.	1-4	0.1	0
	2-7	10	45
	3-7	100	0
M. T.	1	10	+ (delayed)
	2	100	4
V. G.	1	10	22
	2	100	0

*Sites were uniformly prepared on the right upper arm of recipients with 0.05 ml. of undiluted serum from patient I. J.

†The first figure refers to the order number of successive injections of allergenic solution in the left arm of the recipient. The figure following the dash represents the elapsed time in days following the preceding injection; or with injection No. 1 following sensitization.

‡Quantity of cottonseed allergenic protein, CS-13A, contained in 1 ml. of sterile salt solution.

§The numbers refer to the size of the wheal in millimeters produced in the sensitized site. Itching commenced within 5 to 10 minutes, and wheals developed within 15 to 30 minutes.

DISCUSSION

On the basis of generally accepted principles of the relationship between immunologic specificity and chemical constitution, the results obtained can best be interpreted as demonstrating that the principal allergenic component of cottonseed is concentrated in the protein picrate fraction CS-5. CS-5 is considered the major allergenic component because, as judged by wheal size, it produced larger reactions in passively sensitized sites than the residual allergens in unfractionated aqueous extract of cottonseed.

Reagins of one of the sera (Q. N.) were completely neutralized by CS-5 or CS-13A according to the results obtained from both in vivo and in vitro methods of testing. Sera L. C., B. H., and Q. N. appeared to have reagins for CS-5 alone by the indirect method of testing, and

TABLE III

RESULTS SHOWING THE MULTIPLICITY OF ALLERGENS IN COTTONSEED BY IN VITRO NEUTRALIZATION EXPERIMENTS

SERUM*	COTTONSEED PREPARATION*	CONCENTRATION IN UNITS	DUPLICATE SITES TESTED 48 HOURS LATER WITH 100 UNITS OF	
			COTTONSEED UCS	CS-13A
Sher	UCS†	1	4+	4+
	UCS	10	4+	3+
	UCS	100	2+	0
	CS-13A	1	4+	2+
	CS-13A	10	4+	1+
	CS-13A	100	4+	0
	Saline‡		4+	4+
Hough	UCS	1	4+	4+
	UCS	10	3+	3+
	UCS	100	2+	±
	CS-13A	1	3+	3+
	CS-13A	10	3+	2+
	CS-13A	100	2+	+
	Saline		4+	4+
Stein	UCS	10	+	3+
	UCS	100	0	0
	CS-13A	10	2+	2+
	CS-13A	100	0	0
	Saline		2+	3+
M. J.	UCS	1	4+	4+
	UCS	10	3+	4+
	UCS	100	+	0
	CS-13A	1	4+	4+
	CS-13A	10	3+	+
	CS-13A	100	3+	0
	Saline		4+	4+
Q. N.	UCS	10	+	2+
	UCS	100	0	0
	CS-13A	10	±	0
	CS-13A	100	0	0
	Saline		2+	3+
L. C.	UCS	100	2+	0
	CS-13A	100	3+	0
	Saline		4+	4+

*Equal parts of serum and allergen solution in physiologic salt containing the indicated number of units of nitrogen were mixed and allowed to stand at least overnight. Sensitive sites were prepared on normal persons by using 0.1 ml. of the mixture. 1 unit = 0.00001 mg. of protein nitrogen. (Cooke and Stull: J. ALLERGY 4: 87, 1933.)

†UCS, Unfractionated cottonseed extract.

‡Control was prepared by mixing equal parts of serum and saline solution.

the Stein and Q. N. sera possessed reagins for CS-13A alone by the in vitro method employed by Stull.

These results demonstrate the multiplicity of allergens in cottonseed and consequently the presence of multiple cottonseed reagins. Thus, the early observations of Coca and Grove regarding the multiplicity of allergens in cottonseed has been confirmed by isolation of one of the active components and by demonstration of at least one other different allergenic component in the unfractionated cottonseed extract.

SUMMARY

1. The multiplicity of allergens in cottonseed has been demonstrated by the passive transfer technique.

2. The protein picrate, CS-5, has been shown to be the major allergenic component of cottonseed. CS-5 is immunologically distinct from other allergens in cottonseed.

3. Sera from cottonseed-sensitive patients contain a variable number of cottonseed reagins.

The authors wish to acknowledge the assistance of Dorris C. Chambers of the Bureau of Agricultural Chemistry and Engineering in conducting clinical tests.

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THE RELATIONSHIP BETWEEN CLINICAL SYMPTOMS AND POLLEN COUNT IN POLLINOSIS

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ALTHOUGH the parallelism between the intensity of symptoms in hay fever patients and the concentration of pollen in the air has been accepted for a long time, no detailed demonstrable proof of this thesis was offered until recently. In 1937 Waldbott and Ascher[1] presented a graph showing the relationship between the intensity of hay fever symptoms and the daily pollen count. This graph is remarkable for the trueness with which one curve follows the other. Waldbott and Ascher based the construction of their graph on the data collected from 397 hay fever patients. These patients were seen by the authors whenever there were the slightest manifestations of hay fever, and the symptoms were recorded.

In 1934 Feinberg[2] presented in his book a graph illustrating the relationship between the intensity of daily hay fever symptoms and the number of pollen grains per cubic yard of air. This graph indicates corresponding rises and falls for the two curves but not with the same regularity as shown in the graph of Waldbott and Ascher. Feinberg gives no details of the basis for the construction of his curves.

In another paper[3] a report of a study of 122 cases of ragweed and of ragweed and timothy hay fever was made, comparing the results of preseasonal and coseasonal treatment on the one hand with preseasonal treatment alone on the other. Each of these selected patients kept a daily diary of individual symptoms (asthma, eye, nasal obstruction, sneezing). The patients recorded the intensity of these symptoms as 0 (none), 1 (mild), 2 (moderate), and 3 (marked). From these data a curve of individual daily symptoms was constructed and superimposed on a ragweed pollen curve for the same period.

Fig. 1 shows the relationship between the intensity of the symptom, nasal obstruction, and the number of granules of ragweed pollen in the air for each day of the ragweed season. Two symptom curves are shown; the dot and dash line represents the symptoms of the group of patients treated preseasonally and coseasonally, and the broken line represents the group of patients treated preseasonally only. It has already been shown that the results are substantially the same whether patients are treated by the one method or the other.[3]

From the Allergy Division of the Jewish Hospital of Brooklyn.

An analysis of the graph reveals several facts. In a general way a rise in the pollen count is followed by an increase in the severity of nasal obstruction. Thus, following a period of rain on August 17, 18, and 19, the count on August 20 rose, ranging from 20 to 137 granules per 1.8 sq. cm. of exposed slide between August 20 and August 24. This caused nasal obstruction for coseasonally treated patients to rise from 0.51 unit of discomfort on August 19 to a level of 0.78 to 1.26 units of discomfort between August 20 and August 24.

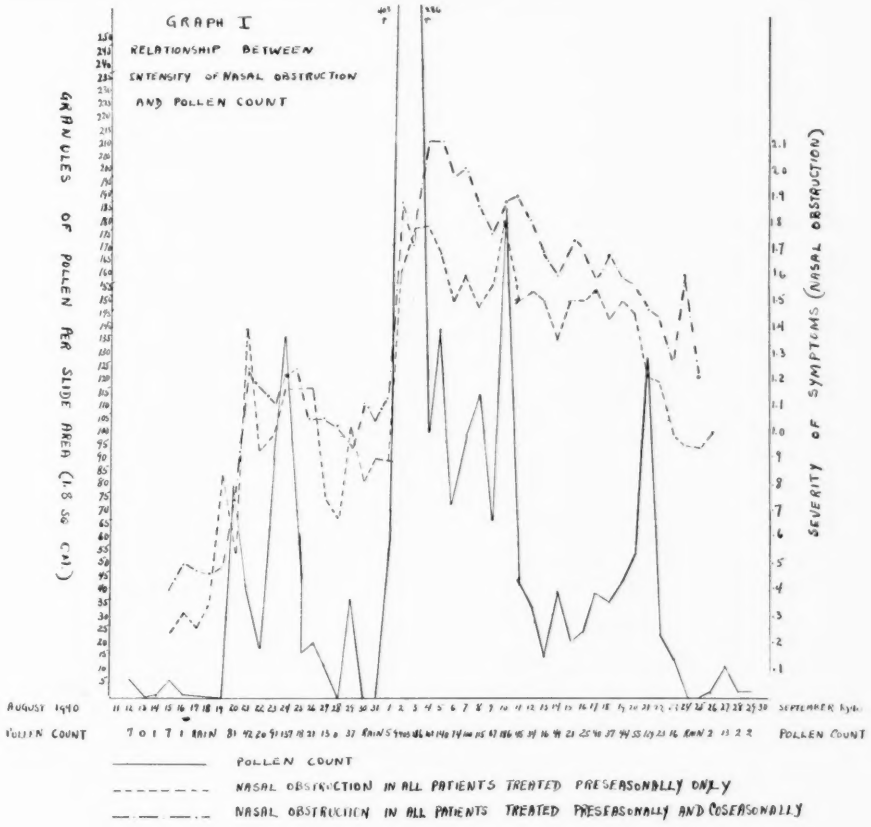
GRAPH I
RELATIONSHIP BETWEEN
INTENSITY OF NASAL OBSTRUCTION
AND POLLEN COUNT
GRANULES OF POLLEN PER SLIDE AREA (1.8 SQ CM.)
SEVERITY OF SYMPTOMS (NASAL OBSTRUCTION)
AUGUST 1940
SEPTEMBER
POLLEN COUNT
POLLEN COUNT
NASAL OBSTRUCTION IN ALL PATIENTS TREATED PRESEASONALLY ONLY
NASAL OBSTRUCTION IN ALL PATIENTS TREATED PRESEASONALLY AND COSEASONALLY

Fig. 1.

The rise in the pollen count and the increase in the intensity of nasal obstruction correlate more closely during the first half of the season than during the latter half. Thus, in passing from August 23 to August 24 there is a 50 per cent increase in the pollen count and a 21 per cent increase in symptoms among coseasonally treated patients. However, in passing from September 9 to September 10 there is a 180 per cent rise in the pollen count with only a 4 per cent increase in nasal obstruction among the coseasonally treated patients.

Sudden rises in pollen count affect the intensity of nasal obstruction more strikingly in the first half than in the second half of the season.

This can be seen in comparing the values for September 1 and 2, with those for September 9 and 10.

Fig. 2 shows the relationship between the intensity of pollen asthma among all patients where this symptom occurred and the pollen curve. Only about forty cases exhibited asthmatic symptoms. The relationship between this symptom and the pollen count is similar to that shown in Fig. 1. A similar relationship between the intensity of sneezing and the pollen count was shown to exist, but the graph illustrating this point is not presented.

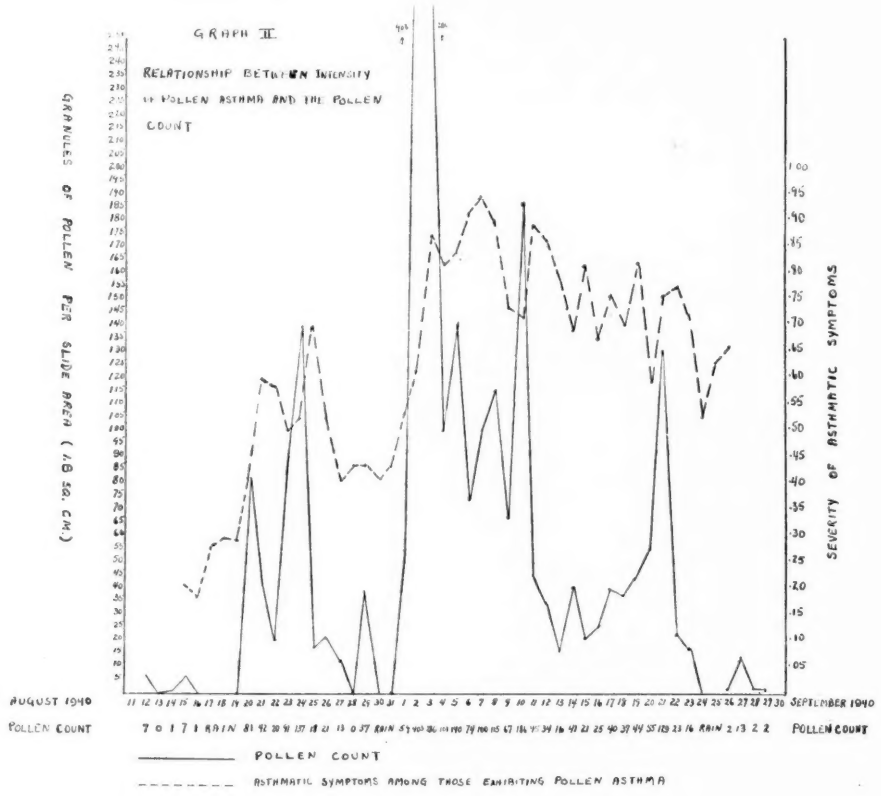
GRAPH II
RELATIONSHIP BETWEEN INTENSITY OF POLLEN ASTHMA AND THE POLLEN COUNT
GRANULES OF POLLEN PER SLIDE AREA (18 SQ. CM.)
SEVERITY OF ASTHMATIC SYMPTOMS
AUGUST 1940
SEPTEMBER 1940
POLLEN COUNT
POLLEN COUNT
POLLEN COUNT
ASTHMATIC SYMPTOMS AMONG THOSE EXHIBITING POLLEN ASTHMA

Fig. 2.

Theoretically, the explanation for the relationships pointed out seems fairly clear. Early in the hay fever season the susceptible mucosae are highly sensitive to stimulation. After being bombarded by pollen, day after day for about three weeks, the shock organs become waterlogged and exhausted. The tissues at this time become relatively refractory to further stimulation. During periods when the pollen count is low, particularly late in the season, symptoms of moderate intensity may still be present because the return of the physiologically disturbed tissues to normal occurs slowly. The longer the duration of the chronic edema, the longer it takes for the tissues to return to normal.

SUMMARY

1. The intensity of the individual symptoms of ragweed hay fever corresponds in a general way to the rise and fall of the pollen curve.

2. The symptomatic fluctuations run much closer to the pollen curve during the first half of the season than during the second.

3. During the second half of the season there is actually a lack of parallelism between the pollen count and the intensity of hay fever symptoms.

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THE VALUE OF CONTINUING PRESEASONAL THERAPY DURING THE POLLEN SEASON

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THE parenteral treatment of hay fever was introduced in 1911.[1] Since that date much has been written on the efficacy of this type of therapy. Authorities still differ, however, as to the most satisfactory methods of treatment to be employed.[2-7] Clinicians disagree on the optimal dosage, the frequency of injections, the number of treatments to be given, and the duration of treatment.

One determinant of results which was of particular interest to us was the time relationship between the therapeutic injections and the pollen season. There is no uniformity of opinion[7] as to whether it is advisable to continue preseasonal treatment into and through the period of active pollination or whether the therapeutic result is just as good or better when specific treatment is discontinued with the onset of pollination. Moreover, those advocating the continuation of treatment coseasonally differ on the amount and frequency of the injections to be given during this period.[3, 9, 10] It was with the hope of shedding some light on this question that we undertook to compare the results obtained with preseasonal and coseasonal treatment with those obtained with preseasonal treatment alone.

One great obstacle to the satisfactory study of the results of any type of hay fever therapy has been the lack of an objective method for evaluating those results.[8] The investigator has been obliged to depend almost entirely upon the statements of the patient for his data. It is hardly necessary to point out the shortcomings of such information, particularly when it involves an estimate by the patient of his symptoms at the end of a six- or seven-week period. This difficulty has, in a good measure, been overcome by us in this study. Our method entails the use by the patient of a daily diary in which the individual symptoms are charted and evaluated separately. The details of this procedure will be described.

The investigation was started on 275 hay fever patients from the allergy clinic of the Jewish Hospital of Brooklyn. However, it was necessary to eliminate those who attended irregularly, those who were not intelligent enough to carry out the instructions for keeping the diary, and those who, in addition to their pollinosis, had symptoms of allergic rhinitis throughout the year. The number remaining at the

From The Allergy Division of the Jewish Hospital of Brooklyn.

close of the study was 122. All of these were cases of pollinosis sensitive to ragweed alone or to ragweed and timothy. The investigation in the latter case was restricted to the ragweed symptoms.

Of the 122 patients, eighty were females and forty-two were males. The family history of allergy was positive in fifty-seven, negative in fifty-five, and unknown in ten. The compiled averages in this series from the point of view of the age of the patient, the age at onset of symptoms, and the duration of illness fell within the range reported by other writers and need not be tabulated here.

All patients were skin tested by the intracutaneous method with a combined giant and dwarf ragweed extract and with a timothy grass extract. Cases were classified according to the degree of their cutaneous sensitivity (Cooke). Injections were given subcutaneously at weekly intervals. The dose was increased each week according to the patient's estimated tolerance and for the most part conformed to the commonly accepted schedule.[9]

Not all patients reached their estimated maximum dose by the onset of the pollen season. The more sensitive patients who attained a maximum dose of 0.02 mg. of nitrogen* or more and the less sensitive patients who attained a maximum dose of 0.05 mg of nitrogen or more were regarded as having received sufficient dosage. All others were considered as having received substandard dosage. Patients receiving less than 0.0125 mg. of nitrogen as a maximum dose were not included in the final tabulations.

On August 15, the beginning of the ragweed season, the patients were divided into two groups. Members of one group continued to receive injections of ragweed pollen extract. The others were given 0.5 c.c. of normal saline subcutaneously at each visit during the pollen season and were not informed of any change in the nature of their treatment.

In order to avoid the production or aggravation of hay fever symptoms by the pollen injections given coseasonally, the final preseasonal dose in each case was halved and repeated at weekly intervals. This was done for the sake of uniformity in procedure despite the fact that some of the patients would have been able to tolerate higher doses.

Seventy-three patients received coseasonal in addition to preseasonal treatment, while forty-nine received preseasonal treatment alone. Fifty-two patients received standard maximum dosage and seventy received substandard dosage. All patients were treated uniformly with regard to medication and dietary restrictions.

The daily record chart employed by the patient is illustrated in Table I. A separate daily space is provided on this chart for each of the symptoms referable to the chest, eyes, and nose, thus facilitating

*Represents milligrams of total nitrogen per milliliter of extract.

the charting and estimation of the severity of symptoms. Patients were instructed to make notations nightly before retiring. Symptoms were graded from 0 to 3, according to their severity, 0 indicating no symptoms, 1, mild, 2, moderate, and 3, marked. At each visit to the clinic the patient's chart was checked to assure accurate recording. At the close of the pollen season the diaries were collected and analyzed. Table II is a sample tabulation of one of the patients, showing the severity of each symptom for the entire period of the hay fever season. A similar tabulation was made for each of the 122 patients from his own diary.

TABLE I

DAILY RECORD CHART FROM AUGUST 15 TO SEPTEMBER 30 INCLUSIVE

No. ----------

Name ----------

	A E S N	A E S N	A E S N	A E S N	A E S N	A E S N	A E S N
August	15	16	17	18	19	20	21
	22	23	24	25	26	27	28
	29	30	31				
September	1	2	3	4	5	6	7
	8	9	10	11	12	13	14
	15	16	17	18	19	20	21
	22	23	24	25	26	27	28
	29	30					

Code.—A, Asthma; E, eyes (itching, burning, discharge); S, sneezing; N, nasal obstruction or nasal discharge. 0, No symptoms; 1, mild symptoms; 2, moderate symptoms; 3, marked symptoms.

TABLE II

SAMPLE TABULATION CHART

SYMPTOM	AVERAGE
Asthma	1.5
Eyes	1.4
Sneezing	1.5
Nasal obstruction	1.6
Average	1.5

In the tables and Fig. 1 the group of patients treated both preseasonally and coseasonally is referred to as the coseasonal or "Co" group, and the group treated only preseasonally is designated as the preseasonal or "Pre" group. The patients in each of these groups in turn were divided into two subgroups according to whether they had received standard (+) or substandard (−) dosage.

In Table III the CO and Pre groups are compared on a basis of severity of all symptoms as a whole, calculated by averaging the individual symptoms. Little difference is noted in the results obtained in the two groups. In Table III comparison is also made between the intensity of all symptoms in the CO+ and Pre+ subgroups and between the inten-

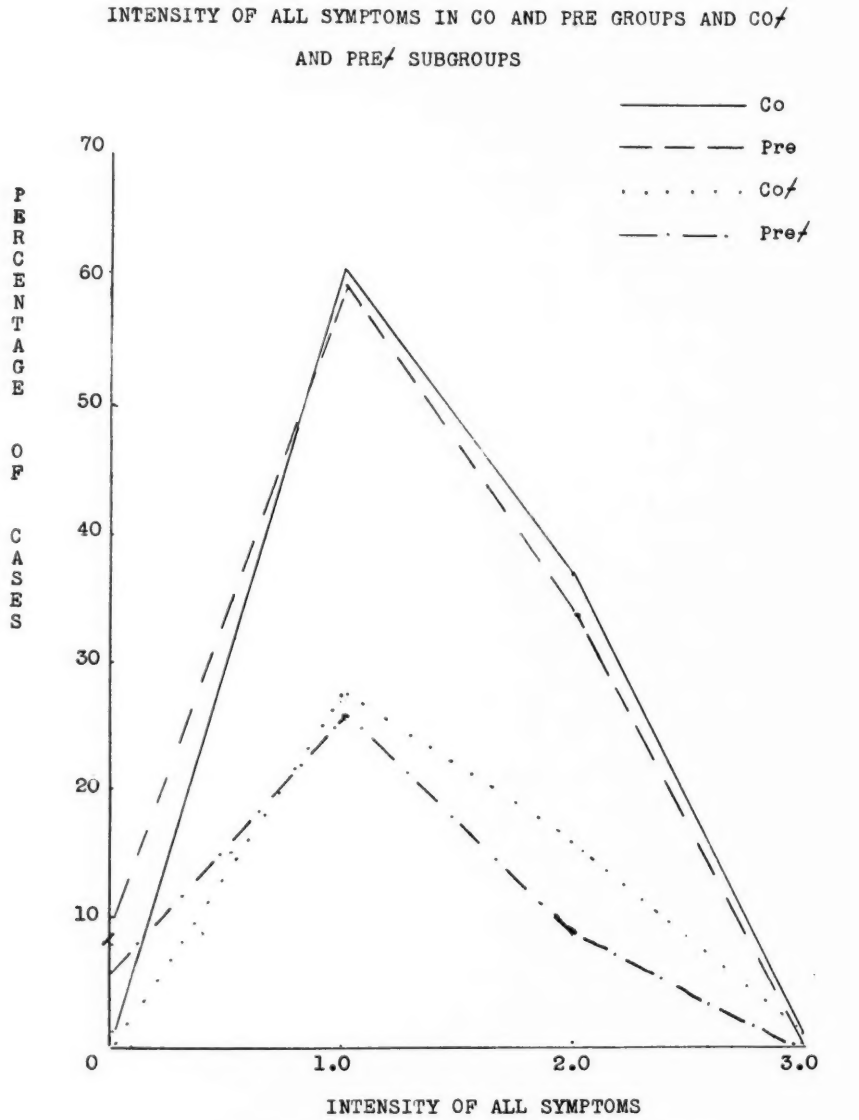
TABLE III

INTENSITY OF ALL SYMPTOMS IN COSEASONALLY AND PRESEASONALLY TREATED GROUPS

INTENSITY OF SYMPTOMS	CO		PRE		CO +		PRE +		CO −		PRE −	
	NUMBER OF CASES	% CASES	NUMBER OF CASES	% CASES	NUMBER OF CASES	%* CASES	NUMBER OF CASES	%† CASES	NUMBER OF CASES	%* CASES	NUMBER OF CASES	%† CASES
0 (None)	1	1.4	4	8.2	0	0	3	6.1	1	1.4	1	2.0
1.0 (Mild)	44	60.2	29	59.2	20	27.4	13	26.5	24	32.8	16	32.7
2.0 (Mod.)	27	37.0	16	32.6	11	15.1	4	8.2	16	21.9	12	24.5
3.0 (Marked)	1	1.4	0	0	1	1.4	0	0	0	0	0	0

*Percentage of Co + or Co − cases is derived by dividing the Co + or Co − cases over the total Co cases.
†Percentage of Pre + or Pre − cases is derived by dividing the Pre + or Pre − cases over the total Pre cases.

sity of all symptoms in the CO– and Pre– subgroups. Here, again, no substantial differences are noted. Where differences do occur they



Co: Cases treated preseasonally and coseasonally
Pre: Cases treated preseasonally only
Co/: Cases treated preasonally and coseasonally with standard dosage
Pre/: Cases treated preseasonally only with standard dosage

Fig. 1.

show a slight advantage (in all instances but one) in favor of preseasonal treatment alone when standard or adequate dosage is given.

These facts are shown in Fig. 1. The curves for Co and Pre as well as for Co+ and Pre+ run almost parallel to one another. The difference in height in each set of curves is slight but indicates some advantage in favor of Pre and Pre+ over Co and Co+, respectively. Reference to Table III shows no difference in the results obtained in the Co– and Pre– subgroups. A comparison between the Co+ and Co– and between the Pre+ and Pre– subgroups reveals that, on the whole, the better results are obtained in the groups in which the patients receive standard or adequate dosage. This is particularly evident in the comparison of the Pre- subgroups.

TABLE IV

INTENSITY OF INDIVIDUAL SYMPTOMS IN COSEASONALLY AND PRESEASONALLY TREATED GROUPS

INTENSITY OF SYMPTOM	ASTHMA				SNEEZING			
	CO		PRE		CO		PRE	
	NUMBER OF CASES	% CASES	NUMBER OF CASES	% CASES	NUMBER OF CASES	% CASES	NUMBER OF CASES	% CASES
0 (None)	48	65.8	33	67.3	0	0	1	2.0
1.0 (Mild)	20	27.4	12	24.5	25	34.2	15	30.6
2.0 (Mod.)	5	6.8	4	8.2	37	50.7	28	57.2
3.0 (Marked)	0	0	0	0	11	15.1	5	10.2
	NASAL OBSTRUCTION				EYE			
0 (None)	1	1.4	4	8.2	11	15.1	5	10.2
1.0 (Mild)	28	38.4	15	30.6	40	54.8	35	71.4
2.0 (Mod.)	32	43.8	27	55.1	20	27.4	8	16.4
3.0 (Marked)	12	16.4	3	6.1	2	2.7	1	2.0

In Table IV the results obtained in the Pre and Co groups are compared on the basis of severity of individual symptoms. Here, too, it may be noted that there is little difference between the results obtained in the Co and Pre groups.

SUMMARY

1. One hundred and twenty-two cases of ragweed hay fever in Brooklyn, N. Y., were studied to compare the effects of the preseasonal and coseasonal therapy on the one hand and preseasonal therapy alone on the other.

2. The results were substantially the same in the two groups.

3. On the basis of total symptoms or of individual symptoms, such as asthma, sneezing, subjective nasal obstruction, and itching of the eyes, both methods of treatment produced similar results.

4. It would appear, therefore, that in this region there is little to be gained by continuing the specific therapy during the pollen season in uncomplicated cases of hay fever which have been properly prepared preseasonally.

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SKIN TESTS AND PASSIVE TRANSFER STUDIES IN NEUROLOGICAL CONDITIONS

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THIS study was made in order to determine the influence of diseases of the nervous system upon the allergic state in the human being. It has been claimed for many years that psychogenic disturbances may be productive of bronchial asthma. Indeed, it is said by some that asthma, eczema, spastic bowel, urticaria, and other abnormal functions are the result of psychogenic influences. More recently the role of the nervous system has received considerable attention. In fact, histamine to which allergic reactions have been attributed by Lewis, produces a flare when injected intradermally, and this flare is entirely dependent upon intact peripheral sensory nerve fibers. It was felt, therefore, that a study of the allergic skin reactions in various types of neurological conditions possibly would elucidate a relationship between allergy and the nervous system.

Inasmuch as the incidence of the allergic state was very small in the available neurological clinical material, the passive transfer method was chosen as a vehicle to study the skin responses. In three of the subjects who were definitely allergic scratch and intradermal tests were used. The influence of induced anesthesia also was observed in a small group of patients. The usual technique was employed, consisting of the intradermal injection of 0.05 c.c. to 0.1 c.c. of serum containing ragweed reagin. After intervals of twenty-four to seventy-two hours 0.02 c.c. of the 1:2,500 dilution of ragweed extract was injected into these sites and the reaction determined. Control sites of ragweed injections were used in all cases. In some of the subjects observations on the effect of intradermal injections of 0.01 c.c. of 1:1,000 histamine were made.

The first group of patients consisted of three allergic persons, two of whom had had ragweed fever for many years, while the third developed asthma after exposure to dog hair. Two of the patients had partial hemiparalysis accompanied by hemianesthesia on the same side. The third patient had a simple hemiparalysis without anesthesia. Ragweed scratch tests and histamine intradermally were applied on the arms, forearms, and back of the normal and abnormal sides of two patients, while, in the third, dog hair was used in place of ragweed. The patient with the hemiparalysis, but no anesthesia, developed a four-plus wheal

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and erythema accompanied by itching in all areas of both sides. The two patients with hemianesthesia and hemiparalysis revealed five- to six-plus reactions on the anesthetized side compared to two- to three-plus reactions on the normal side. In one of these patients a gradual return of sensation over a period of months was accompanied by changes in skin response which became almost equal on both sides. It would seem that cerebral lesions involving loss of function of sensory fibers result in increased allergic skin responses on the involved side, but, as sensory function returns, equalization of skin responses follows.

TABLE I

SKIN RESPONSES IN ALLERGIC PATIENTS WITH NERVOUS SYSTEM PATHOLOGY

TYPE OF CASE	INVOLVED AREA		NORMAL AREA	
	SCRATCH TESTS	HISTAMINE	SCRATCH TESTS	HISTAMINE
Ragweed hay fever; right hemianesthesia; right partial hemiparalysis	+++++		+++	
One month later (anesthesia much less)	++++		+++	
Dog hair sensitive; right hemianesthesia; right partial hemiparalysis	++++	++++	++	++
Ragweed hay fever; left hemiplegia; no anesthesia	++++		++++	

The second group of patients comprised the nonallergic including the usual type seen in the neurological ward. There were sixteen different kinds of neurological conditions which could be sensitized passively without any more difficulty than the normal nonallergic patient. These included paralysis agitans, syringomyelia, general paresis, brain tumor, gunshot wound of brain, poliomyelitis, cerebral hemorrhage, alcoholic neuritis, multiple sclerosis, amyotrophic lateral sclerosis, Recklinghausen's disease, cerebrospinal syphilis, tumors of cord, fractured cervical vertebrae, arsenical neuritis, and dementia praecox. Many of these were aged patients and undernourished. Some showed mental deterioration, while others presented paralyses and atrophies. Their responses to passive transfer were as definite as noted in the normal.

Of particular interest were five patients with cord tumors and three others with fractured cervical vertebrae. Passive transfer reactions were of varying intensity in all areas above and below the lesions. The histamine responses, however, showed a decreased skin reaction in the areas of anesthesia. In other words, the histamine flare, although present throughout, decreased in proportion to loss of skin sensation. In one case a repetition of the experiment a year later again showed equal and positive passive transfer reactions in all areas. The histamine

responses, however, in this subject did not present decreased reactions in the anesthetized areas as before and were practically the same at all sites. This may have been due to partial return of sensation. Two of the cord tumor subjects revealed dermographia in normal skin areas not apparent in anesthetized skin.

TABLE II

PASSIVE TRANSFER AND HISTAMINE IN SPINAL CORD PATHOLOGY

TYPE OF CASE	INVOLVED AREA		NORMAL AREA	
	PASSIVE TRANSFER	HISTAMINE	PASSIVE TRANSFER	HISTAMINE
Angioma cord with paraplegia and anesthesia (thighs)	++++		++++	
Cord tumor; paraplegia; no anesthesia	+++		+++	
Cord tumor; anesthesia, right side	++++		++++	
Chondroma; paraplegia; anesthesia below third rib	+++	++	+++	++++
Cord tumor, second thoracic; paraplegia; anesthesia below third rib	+++	++	+++	++++
Fracture cervical vertebrae; anesthesia below clavicles; paraplegia	+++	+	+++	++++
Fracture cervical vertebrae; anesthesia below second rib; paraplegia	+++	+	+++	++++
One year later	+++	+++	+++	++++
Fracture cervical vertebrae; (three days after fracture); anesthesia below second rib; paraplegia	++++		++++	

The third group of patients consisted of those in whom spinal anesthesia, nerve block, and local infiltration anesthesia were used. Passive sensitization was effected prior to operation, while ragweed and histamine injections were done during spinal anesthesia. In every instance histamine flare and passive transfer responses, although never absent entirely, diminished in proportion to the degree of anesthesia. Injections of histamine and ragweed into sensitized areas after anesthesia had disappeared resulted in good responses, as seen in normal unanesthetized skin. Histamine injections into sensitized areas under anesthesia rendered these sites almost entirely refractory to ragweed after anesthesia was worn off.

In two patients 2 per cent novocain infiltration of the forearm was done, and in one other the brachial plexus was blocked. Comparison of the normal and anesthetized arms and forearms revealed no apparent difference in passive transfer and histamine response. Apparently spinal anesthesia produces changes in histamine and passive transfer reactions not evident in local infiltration anesthesia and peripheral nerve block.

In six patients in whom sympathectomy had been performed for Raynaud's disease of the extremities there was a normal response to passive transfer tests. Division of the sympathetic nerve fibers did not influence passive transfer response observed in a normal nonallergic person.

TABLE III

PASSIVE TRANSFER AND HISTAMINE UNDER INDUCED ANESTHESIA

TYPE OF ANESTHESIA	ANESTHETIC AREA		NORMAL AREA		REMARKS
	PASSIVE TRANSFER	HISTAMINE	PASSIVE TRANSFER	HISTAMINE	
Local infiltration of forearm	++	+++	++	++++	No histamine flare in novocain area
Brachial plexus block	++	++	++	++	Histamine before ragweed induces refractory passive transfer to ragweed
Spinal anesthesia	+	+	++++	++++	Reactions refer to amount of flaring

There are a number of responses which seem paradoxical. The passive transfer and histamine reactions in the anesthetized areas induced by spinal anesthesia revealed decidedly diminished reactions, whereas in the hemianesthetic side of the allergic patients a definite increase to histamine and scratch ragweed applications was observed. Apparently the release of direct cerebral influence in the sensory tracts produces peripheral vasomotor changes which enhance the allergic skin responses. Whether the variation in response can be attributed to differences in the amounts of acetylcholine liberated or whether it is due to local vasomotor changes or other factors is difficult to state. A comparison of the decreased reactions of the areas under spinal anesthesia to those induced under peripheral nerve block and infiltration anesthesia suggests that, although in spinal anesthesia the principal action is on the nerve roots before they leave the dura, there may also be sufficient anesthesia of the cord to alter skin responses. There are also variations in passive transfer and histamine responses in the cord cases to suggest differences in the mechanism.

Intradermal histamine injections are being used as a diagnostic measure to determine the presence and location of peripheral nerve lesions which do not produce a flare response. In two of the cord cases dermographia was noted. When the skin was stroked with a pin, there was a lack of dermographia in the anesthetized areas, but, as the normal skin was approached, increased dermographia in proportion to sensation was noted. The dermographia paralleled the histamine responses in the cord cases with the exception that the whealing and flare following stroking of the anesthetized areas seemed to be absent or barely perceptible. The histamine reactions presented definite whealing and flare but were much reduced.

The general group with neurological conditions were passively sensitized as readily as were normal subjects. The degree of mental excitement or depression did not seem to influence the intensity of passive transfer as seen in normal subjects.

SUMMARY

1. Allergic patients with hemianesthesia gave increased reactions to scratch and intradermal tests and histamine on the affected side. Hemiparalysis did not influence the skin responses to these tests.

2. Passive transfer in sixteen stated types of neurological conditions was as effective as in the normal.

3. Local infiltration anesthesia and nerve block did not alter passive transfer and histamine responses. Spinal anesthesia reduced passive transfer and histamine responses in proportion to the degree of anesthesia.

4753 Broadway

Special Reports

SEITZ FILTERING DIRECTLY INTO STORAGE BOTTLES: TWO METHODS

DAVID M. PIPES, M.D.
GREENSBORO, N. C.

FILTERING extracts and other materials directly into permanent containers by use of the Seitz apparatus presents several advantages. (1) The procedure requires less time than the old method, in that it eliminates the necessity of transferring the filtered material from the filtering apparatus to sterile bottles. (2) There is less likelihood of contamination, as there is proportionately less handling of the apparatus and Seitzed material by the operator. (3) The direct method of filtering employs a less complicated and more economical apparatus.

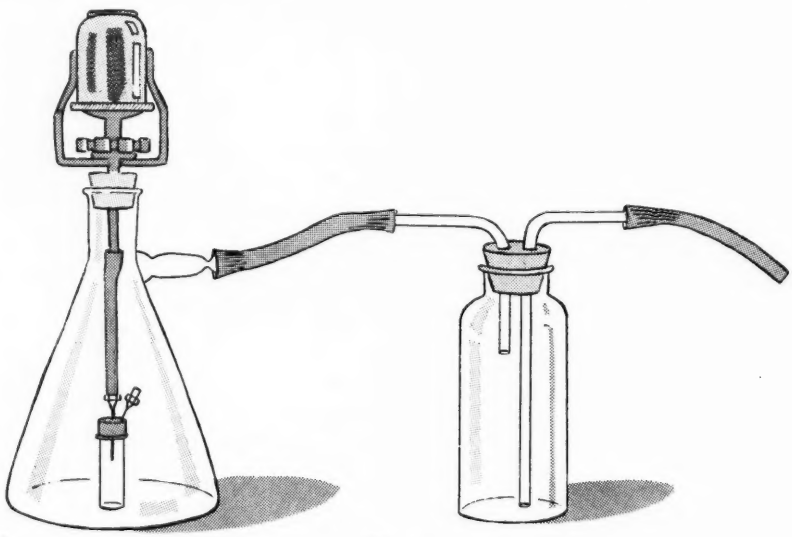

Fig. 1.

The first method is used for small quantities of material for which small storage bottles or vials are suitable. This employs negative pressure or suction as a filtering force. The small bottle or vial is placed inside the Erlenmeyer flask, having been connected to the Seitz filter by a length of rubber tubing, as shown in Fig. 1. A 23 gauge needle pierces the middle of the bottle cap and is used as an inlet. A short

26 gauge needle stuck through the rubber cap along its edge is used to transfer negative pressure from the Erlenmeyer flask into the small bottle. The apparatus is connected in turn to a trap bottle and suction pump.

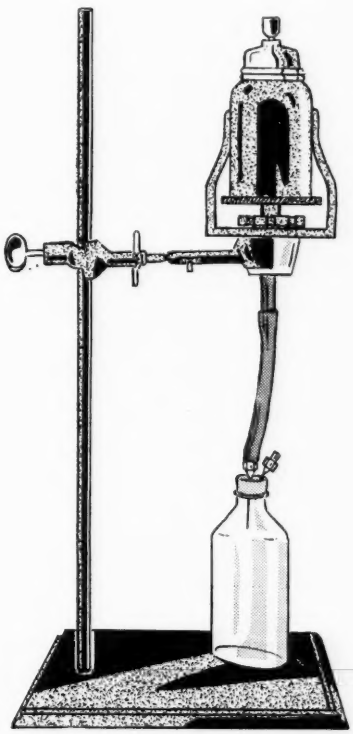

Fig. 2.

The second method is used for larger quantities of material requiring larger storage bottles, and employs positive pressure as a filtering force. The apparatus is set up as shown in Fig. 2. Small elastic bands may be used to reenforce the rubber tubing connections.

At the completion of the filtering process by either method the filtered material is under atmospheric pressure in its container and ready for permanent storage.

With the exception of the storage bottles or vials, the filtering apparatus, with or without Erlenmeyer flask, may be autoclaved in one unit.

101 North Elm Street.

ALLERGY TO AUSTRALIAN PINE

A Report of Three Cases

Nelson Zivitz, M.D.
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IN FEBRUARY, 1940, coincident with the observation of an unknown pollen on exposed slides, three patients developed typical symptoms of hay fever and asthma. A botanist first identified this pollen as hackberry. However, it soon developed that there are no hackberry trees in this area. Further investigation since that time proved the pollen to be *Casuarina equisetifolia* and *Casuarina lepidipholia,* two members of the tree family commonly known as Australian pine. This pollen was not found in the last survey made in the Miami area in 1930.[1]

There are fifty-five known and listed species of Australian pine, the most common being *Casuarina equisetifolia.*[2] Despite its name it is not a conifer like the true pine. The *Casuarina* group is classed near the walnut or hickory tribes, but their real place is uncertain. There are many common names for this tree, but those most often used are Australian beefwood, Australian oak, swamp oak of Australia, and Australian pine. The tree predominates in Tasmania and is also found in Australia, Polymesia, India, South America, and the southernmost part of North America. It was imported to Florida from Cuba about fifty years ago and within the past few years has been planted extensively about Miami Beach, Hialeah, and Homestead on the east coast of Florida. It has also been planted recently in California. The tree grows well in poor, brackish soils and sometimes reaches a height of 200 feet which makes it useful as a windbreak for the protection of citrus orchards.

It is of importance to note that the pollen of *Casuarina equisetifolia* and *Casuarina lepidipholia* can be readily distinguished. Very often both species pollinate at the same time, and the skin reactions are interchangeable. Through the courtesy of Dr. C. H. Dickson, secretary of the Victoria branch of the British Medical Society, we were able to obtain a sample of pollen from *Casuarina suberosa,* a species found in Victoria, Australia. Only one patient (M.Z.) was available for a scratch test with this material, and the reaction was positive.

When this pollen was first found on exposed slides in February, 1940, it had a concentration of 13 to 20 granules per 1.8 sq. cm. per twenty-four hours. It continued to be present in the air during the early part of March and then again from the first to the fourteenth of April. The

greatest concentration was noted in April and on the days when there was a southeasterly breeze.

The following year *Casuarina* flowered much earlier, and the pollen grains were first found on the slides on December 30. The highest concentration was again noted in April, and the pollen was frequently absent when there was a direct easterly ocean breeze. The peak was on April 14, but pollen grains were found until April 28. On the peak day the pollen count was 72 granules in twenty-four hours. Mr. Alexander at the University of Miami states that yearly variations in the pollination of this tree will occur depending upon prevailing atmospheric conditions.

The only previous reference to this tree in the literature is contained in an article by Metzger.* He stated that November is the season of its heaviest pollination in the Tampa area and that many patients were tested with extracts, but positive results have not yet been obtained. Durham[3] stated that there is no reason why its pollen should not cause trouble. This difference in designating the peak month of pollination in Miami and Tampa may be explained by a difference in the species of the respective areas. *Casuarina cunninghamia*, which is rather sparse in Miami, pollinates here in late October. There are large numbers of this species on the west coast and in central portions of Florida. It is therefore not a problem in Miami purely because it is not abundant. It is, however, antigenic and wind borne.

TABLE I

	M.Z.	F.S.	F.P.
Scratch test	Marked	Marked	Moderate
Intradermal test 10 u.	Marked	Slight	Doubtful
Intradermal test 100 u.	Marked	Moderate	Moderate
Intradermal test 500 u.	Not done	Marked	Marked
Passive transfer	Positive	Positive	Not done
Nasal test	Positive	Not done	Not done

The three patients in this group identified the onset of their symptoms with the appearance of the pollen on the slides. However, one patient (F. P.) had symptoms only on days when the pollen counts were high; consequently he was ill in April, and during the remainder of the time he was well. All three patients have made their homes in Miami Beach for at least six months of the winter season each year for two to five years. Each patient had suffered from either hay fever or asthma or both in his home city in the North, and all were completely well for at least the first two winters they spent on Miami Beach. During the remainder of the year, when *Casuarina* was not found on the slides, these patients were free from symptoms, although in the same locality in Florida.

*Dr. Metzger in a personal communication informed me that since his last article was published, he has had a male patient aged 25 years with a history of asthma that is perennial but definitely exaggerated in the latter part of October and through November. Examination showed multiple sensitivities. A positive direct and a positive passive transfer were obtained with extracts of Australian pine. Relief from treatment was very marked.

All three patients gave positive reactions to scratch tests made with dry pollen and to intracutaneous tests with a dilution of 100 protein nitrogen units per cubic centimeters. One patient had a marked positive reaction to the dilution of 10 P.N.U. Specific reagins were demonstrated in two of the patients by the passive transfer test. This test was not done on the third patient. Only one patient was available for an out-of-season nasal test done with dry pollen. In this patient the test was definitely positive.

An extract has been prepared, and hyposensitization is now being attempted. One patient (M.Z.), when the dose of 50 P.N.U. was reached, had a marked local reaction with mild sneezing and headache. At the same time the test site on the forearm, which had been made several weeks before, became red and itched.

SUMMARY

1. *Casuarina equisetifolia* and *Casuarina lepidipholia,* commonly known as Australian pine, pollinate in Miami Beach during January, February, March, and April. The height of the pollinating season for the past two years has been in April.

2. The pollen of *Casuarina* is abundant, wind borne, and capable of producing hay fever or asthma in allergic persons.

3. Three patients studied had hay fever, asthma, or both in the North but not during their first two winters in Miami Beach. In the subsequent winters in Miami Beach, all three patients had hay fever or asthma at intervals during January, February, March, and April.

4. From a study of atmospheric slides during the past two years, it was found that symptoms appeared and disappeared concurrently with the presence or absence of the *Casuarina* pollen in the air.

5. Positive scratch tests and positive intracutaneous tests were obtained on all three patients. Specific reagins were demonstrated in two of the patients. A nasal test with dry pollen done out of season on one patient was positive.

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2. Robinson, W. D.: Editor, Flora and Sylva, London, 1904, vol. 2.
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A SLIDE FOR COUNTING ATMOSPHERIC POLLEN

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During the prosecution of a study on the atmospheric pollen content of Minas Gerais, Brazil, we had difficulty in adjusting our microscopes so that the lens covered a field of 1.4 mm. in diameter, as advised by Vaughan.[1]

In order to simplify the technique, we had the common microscopic slide frosted leaving in its center a surface area of 3.61 sq. cm. (Fig. 1). This represents an area twice as large as that commonly employed, i.e., twice 1.8 sq. cm. In order to have uniformity and a basis for comparison with pollen surveys carried on by other investigators, we divided the number of pollen granules counted by 2, recording the results in the standard area.

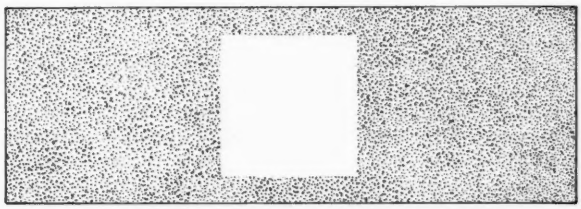

Fig. 1.—Slide for counting atmospheric pollen.

The area so constructed is advantageous because it permits taking the average of the counting, as though we had counted two slides. Besides this, the frosted slide offers the following advantages. The examiner does not have to count the number of the short width of the slide he has traversed, as the frosted part of the slide perfectly limits the area to be covered, without having to adjust the microscope barrel. Any microscope will do for the counting. On the frosted part one may write (with a pencil) the date, place, or other observation of interest in case the slide is to be kept or sent from one place to another. The frosted part holds the jelly in place, avoiding its slipping.

We have found this slide very useful to send to colleagues in various cities of Brazil who cooperate with us.

REFERENCE

1. Vaughan, W. T.: Practice of Allergy, St. Louis, 1939, The C. V. Mosby Co., pp. 469-470.

From the Department of Allergy of "Laboratorio Carlos Chagas," Belo Horizonte, Brazil.

Selected Abstracts

Asthma and Hay Fever

UNDER THE DIRECTION OF SAMUEL M. FEINBERG, M.D., AND
THEODORE T. BERNSTEIN, M.D., CHICAGO

Pollen and Mold Survey of Southeastern New England, 1940. Pratt, H. N., Colmes, A., Fromer, J., Greene, J. E., Chaffee, F. H., and Clapp, W. B.: New England J. Med. **225**: 533, 1941.

For the purpose of studying the variations of daily counts both within Boston and nearby stations, pollen and mold studies were made in 1940 from six stations, three of which were in Boston, one in Worcester, another in Providence, and a third in suburban Boston. Daily twenty-four-hour slide and thrice weekly Petri dish exposures were made. There were found to be three clearly defined pollen seasons for southeastern New England: trees from April 19 to May 30, grasses from early June to mid-July, and weeds from August 20 to late September. The Alternaria spore season overlaps the grass and weed seasons and persists later. Grass and weed pollens were found to be more abundant in the suburban areas than in the city, and tree pollen was equally abundant in both places. Pollen peaks at neighboring stations were not always consistently superimposed, which demonstrates the effect of local conditions, such as wind currents and vegetation, upon the counts.

A Comparison of Ball-Mill and Coca Extracts of Ragweed Pollen in the Treatment of Hay-Fever. Winkenwerder, W. L., Arbesman, C., Gay, L. N., and Eagle, H.: Bull. Johns Hopkins Hosp. **69**: 287, 1941.

The authors treated by the preseasonal method a group of fifty-seven ragweed hay fever patients with an extract made of short ragweed pollen granules which had first been disrupted in the ball mill. The results of treatment in this group were as good as, but not superior to, the results attained in a control group of fifty-seven ragweed hay fever patients who had been treated during the same season with a buffered extract (Coca solution) made from the defatted but intact cells of short and giant ragweed pollen. This is of particular significance in view of the fact that the ball mill extract contained twice as much protein nitrogen as did the Coca extract and consisted wholly of short ragweed pollen, which is reputed to have greater activity than giant ragweed pollen. Also most of the patients treated with larger amounts of extract were in the group receiving the ball mill preparation.

Experimental Asthma: Treatment With Histaminase. Neely, F. L.: J. Lab. & Clin. Med. **27**: 319, 1941.

Guinea pigs sensitized to egg white by intraperitoneal injection react to the inhaled vaporized antigen in a manner very similar to bronchial asthma in the human being. The symptoms come on in a few minutes and are characterized by sneezing, prolonged scratching of the nose, increased respiratory rate with prolonged expira-

tions, coughing, and dyspnea. Musical whistles and squeaks can be heard with the stethoscope. The attack usually subsides in about thirty minutes after withdrawal from the chamber. Because the animals always reacted in such a predictable manner after exposure to the inhaled vaporized egg white and never showed signs of anti-anaphylaxis or desensitization even after repeated exposures, it was felt that allergic asthma in guinea pigs would be an excellent method of measuring the effect of histaminase on the reaction. Three units of histaminase given intravenously had no effect in preventing or lessening the severity of an asthmatic attack caused by inhalation of vaporized egg white 15 minutes after the histaminase injection. To detect a delayed effect of the histaminase, some of the guinea pigs were sprayed again in twenty-four hours, but they again reacted with asthma of the same degree of intensity. Three of the animals which again received histaminase three weeks after an initial injection developed symptoms of shock of an anaphylactic nature. This appears to be a true sensitization to histaminase.

Pollen Disease in the Absence of Positive Skin Tests. Colmes, A.: New England J. Med. **225**: 817, 1941.

This report deals with that small group of patients with pollen disease who fail to give positive skin reactions to the prevailing pollens and molds. In the author's experience this group constitutes 7 per cent of the total pollen cases he has treated. In the absence of positive reactions, the diagnosis is based on the history of symptoms occurring during the pollen season and their intensity fluctuating with the variations in pollen pollution. In addition, this group reveals an incidence of positive family history, past history of allergy, and blood eosinophilia comparable with similar findings in other types of human hypersensitiveness. The other clinical characteristics, such as the response to specific therapy and the occurrence of systemic reactions, leave no doubt that this group of patients are true pollen cases. Treatment was carried on with the pollen extract corresponding to the pollen in the air when symptoms were present, the dosage being rapidly increased. This may account for the fact that there were six constitutional reactions in a group of nineteen patients despite the lack of positive skin reactions. It is suggested that the tolerance of the "shock organs" does not correspond to that of the skin, for most of the systemic reactions occurred in the absence of local reactions. On the whole, good results were attained, as fifteen of the nineteen patients benefited by the treatment.

Immunology

UNDER THE DIRECTION OF MATTHEW WALZER, M.D., AND MURRAY ALBERT, M.D., BROOKLYN

Anaphylaxis. Dragstedt, C. A.: Physiol. Rev. **21**: 563, 1941.

In an extensive critical review of the literature on anaphylaxis, the author attempts to arrive at a satisfactory hypothesis as to the pathogenesis of the symptoms. The symptoms commonly observed in anaphylaxis are contraction of smooth muscle, incoagulability of the blood, leucopenia, and a liberation of histamine from the tissues. The incoagulability of the blood observed in dogs and other animals during anaphylactic shock is adequately accounted for by the liberation of heparin. There is no experimental evidence, however, that heparin causes the other symptoms of anaphylaxis, since the injection of heparin in animals fails to produce any ill

effects. The leucopenia which occurs in some animals during anaphylaxis is apparently due to a marked contraction of the arterioles and the formation of leucocytic emboli which block the circulation in the capillaries and veins. The liberation of histamine accounts for all the other symptoms of anaphylaxis. Apparently choline is also liberated during shock in some animals. However, the role which it plays in the production of symptoms is controversial. From the experimental evidence recorded in the literature, the author concludes that the chief symptoms of anaphylaxis may be adequately explained as an autointoxication by physiologically active substances normally present in various tissue cells and discharged from them because of change in cellular permeability resulting from the reaction between antigen and antibody. The reactions resulting from anaphylatoxins may occur in the tissues from which they were liberated or in remote organs or tissues.

Hereditary Predisposition to Sensitization in Guinea Pigs. Jacobs, J. L., Kelley, J. J., and Sommers, S. C.: Proc. Soc. Exper. Biol. & Med. **48**: 639, 1941.

Experimental studies on the inheritance of allergy have been retarded by the need of a sensitizing agent which would clearly indicate the differences in members of a species in their ability to be sensitized. Fifty-seven guinea pigs were treated by the application of a drop of mustard oil, allyl isothiocyanate, to the skin of the back. Applications were made twice a week for from three to five weeks. At the end of this period the animals were tested for sensitivity by the application of a 5 per cent solution of mustard oil to the skin of the flank. Six animals in the group showed moderate to strong reactions and five others showed weak, positive reactions. Further treatment failed to increase the intensity or incidence of sensitization. Selective breeding experiments were undertaken with the animals which were sensitized to allyl isothiocyanate. A strain of guinea pigs was developed, all the members of which could be easily and strongly sensitized to allyl isothiocyanate. These animals did not show an increase in nonspecific reactivity to the testing solution before sensitization. The sensitization to allyl isothiocyanate was specific. An hereditary predisposition to sensitization in guinea pigs was therefore clearly demonstrated.

Studies in the Release of Histamine From the Blood Cells of the Rabbit by the Addition of Horse Serum or Egg Albumen in Vitro. Rose, B., and Browne, J. S. L.: J. Immunol. **41**: 403, 1941.

Samples of whole blood obtained from a group of rabbits which had been sensitized with horse serum or egg white were heparinized. Small amounts of 3 per cent egg albumen, horse serum, or saline solutions were added to the blood samples and the mixtures were incubated at 38° C. for twenty minutes. The histamine content of the plasma was assayed by the Code modification of the Barsoum and Gaddum method. An appreciable increase in the histamine content of the plasma was noted in mixtures which contained the specific antigen with which the animal was sensitized. The addition of horse serum or egg albumen to blood of unsensitized controls produced an increase in the histamine content of the plasma, but this was never so great as that produced when the related antigen was added to the blood of the sensitized animal. Egg albumen, for some unexplainable reason, produced a greater rise in the plasma histamine of unsensitized animals than horse serum. These experiments indicate that histamine is released from the blood cells into the plasma in anaphylactic and anaphylactoid reactions in rabbits. The histamine hypothesis alone, however, does not fully explain the mechanism of anaphylactic shock in the rabbit.

Acute Local Anaphylactic Inflammation of the Lungs. Cannon, P. R., Walsh, T. E., and Marshall, C. E.: Am. J. Path. **17**: 777, 1941.

Purified egg albumen solution was instilled intranasally in rabbits previously sensitized either actively or passively to this antigen. At varying intervals following this procedure the animals were killed, and sections of the pulmonary tissue were examined microscopically. Only minor degrees of pulmonary inflammation were noted in nonsensitized animals. In sensitized animals, however, marked pulmonary inflammation was found, consisting of alveolitis, bronchitis, edema, consolidation, acute arteritis, dilatation of the perivascular lymphatics, phlebitis, and mural thrombosis. Another series of rabbits was passively sensitized by the intranasal instillation of rabbit-antihorse serum and subsequently subjected to intranasal instillations of normal horse serum. Microscopic examination of the pulmonary tissues of these animals also showed evidence of severe inflammation. These findings indicate that antigen-antibody reactions resulting in increased capillary permeability and severe vascular injury occur in the lungs of sensitized animals. The vascular phenomena are due to the presence of anaphylactic antibodies and are not the result of a changed reaction capacity of the tissue cells, since the local anaphylactic reaction is the same in both actively or passively sensitized animals.

Concerning the Mechanism of Anaphylactic and Tryptic Shock. Silva e Rocha, M.: J. Immunol. **40**: 399, 1941.

The addition of trypsin to the Dale bath produces a contraction of the uterine strips of a sensitized guinea pig. The reaction is similar to that observed when the specific antigen is added. Shocking the uterine strip by the addition of the specific antigen does not inhibit a subsequent response to trypsin and vice versa. Blood pressure studies made on sensitized rabbits also indicate that the actions of trypsin and the specific antigen are independent of each other. The similarity in the action of trypsin and specific antigen may be in the capacity of both to liberate histamine from the tissues. Since trypsin and the specific antigen do not desensitize tissues against each other, the site of action is probably not the same in the two mechanisms.

Organic Specificity of Tissues of the Dog and Man as Shown by Passive Anaphylaxis in Guinea Pigs. Bailey, G. H., and Raffer, S.: J. Exper. Med. **73**: 617, 1941.

Rabbits were sensitized with heat-killed vaccines which had been grown in infusion broths made from various tissues of dogs and man. The rabbit immune sera, containing antibodies for both the broths and the organisms, were used to sensitize guinea pigs passively. The passively sensitized animals were tested by means of intravenous injections of autoclaved extracts of the canine and human organs. Severe or fatal anaphylaxis resulted when broths made from homologous organs were injected. In some instances, the injection of heterologous organ extracts of the same species also produced fatal anaphylaxis. The antisera of animals immunized with broths made of striated muscle, ileum, kidney, placenta, and fibromyomas showed the highest degree of specificity. Antisera against whole blood, liver, and lung broths were least specific. Animals tested with broths of heterologous tissues were not desensitized to homologous tissue broths. These experiments point to the presence of thermostable, water-soluble, organ-specific substances in the various tissues of man and dog.

Effect of Histaminase Pretreatment on Histamine Shock in Guinea Pigs. Rose, B., and Browne, J. S. L.: J. Immunol. **41**: 409, 1941.

Karady and Browne had previously found that pretreatment of guinea pigs with intravenous injections of histaminase markedly reduced the intensity of histamine shock. In the present study, these experiments were repeated. A series of guinea pigs were placed into three groups and the dosage of either histamine or histaminase solution was varied in each. The histaminase was administered intravenously fifteen minutes prior to the intra-abdominal injection of the histamine. The pretreatment with histaminase failed to alter the symptoms or severity of histamine shock in these guinea pigs. Hence, the findings of Karady and Browne could not be confirmed and the conclusion was drawn that in these studies histaminase was not active in vivo.

Pediatrics

UNDER THE DIRECTION OF WILLIAM C. DEAMER, M.D., SAN FRANCISCO

Heteroallergy: Parallergy and Metallergy. Urbach, E.: Arch. Pediat. **58**: 780, 1941.

The following author's summary serves best to describe this article:

"The term heteroallergy is used to designate the altered reactive capacity of a specifically allergized organism to other or 'hetero' allergens. Heteroallergy can be subdivided into two basic types: Parallergy and metallergy, either of which can be manifested as a condition of hyper- or hyposensitiveness."

The term "parallergy" is employed to designate the following observation: that a specifically sensitized organism, during the development of an allergy or at a time of considerable fluctuations in the allergic condition, will react to other antigens with allergic manifestation clinically different from the manifestations elicited by the original sensitizing allergen.

The term "metallergy" is used to denote the following fact: that a specifically sensitized organism, one in which the allergic condition is usually either very mild or no longer present at all, will respond to subsequent exposure to antigens of another type, with specific allergic reactions, presenting the same clinical picture as elicited by the first allergen.

Introduction of the concepts "pathergy" and "metallergy" serves to explain a number of recognized but hitherto not satisfactorily explicable phenomena of hypersensitiveness.

Thus, parallergic hypersensitiveness explains how previously negative tuberculin, Schick, and Dick reactions become positive at the time of maximum reaction to vaccination or disease (such as tonsillitis, encephalitis, etc.). It also explains the fact that in an organism which has been allergized by an infection, and particularly by a focal infection, otherwise harmless saprophytes can become pathogenic and lead to acute appendicitis, cholecystitis, pyelitis, etc.

Parallergic hypo- and insensitiveness are the reasons that during the eruptive stage of acute diseases (e.g., measles), the previous reactivity to other antigens (e.g., tuberculin) is generally lost.

Metallergic hypersensitiveness serves to explain the flare-up of allergic reactions, such as an allergic contact dermatitis following exposure to apparently nonspecific agents; the appearance of positive cutaneous reactions in, for example, a tuberculous individual following injection of coli vaccine, etc.; the pathogenesis of infantile

eczema; the transformation of specific allergy into a polyvalent metaspecific allergy (metallergy) and then finally into a nonallergic pathergy, a mechanism commonly referred to as "broadening of allergic base"; the specific action of such apparently nonspecific measures of treatment as injections of tuberculin, peptone, etc.

Metallergic hypo- and insensitiveness underlies the diminished reactivity to unrelated antigens during the course of a specific hyposensitization therapy.

Dermatitis Due to "Antiseptic Oils." Lapin, J. H.: Am. J. Dis. Child. **63**: 89, 1942.

In six children under 3 months of age, the area on the face where antiseptic baby oils had been applied was the site of an erythematovesicular, occasionally papular, dermatitis. Upon omission of oil the lesions completely disappeared in a few days. Patch tests with the oils in question were positive in all six cases after forty-eight hours, while controls of olive oil and petrolatum remained negative.

Thirty newborn infants under 10 days of age were then tested. One gave a positive patch test. Controls of "salad oil," presumably cottonseed oil mainly, were negative.

The offending substance was presumed to be hydroquinone which is extensively used in oils of this type as an antioxidant. Hydroquinone is known to have a high index sensitivity from a dermatologic standpoint and to be toxic on ingestion.

Since it is common practice during the newborn period to give daily rubs to the entire body with antiseptic baby oils containing hydroquinone, the question is raised of the possibility of sensitization to hydroquinone occurring in this manner.

Control of Juvenile Asthma by Vaccine Therapy. Moody, E., and Howard, W. M.: Arch Pediat. **58**: 774, 1941.

Two types of streptococcus vaccine were used therapeutically for asthma in children, one derived from individuals having a common "cold," the other from individuals having the "recurrent type of respiratory disease we call influenza." Both were prepared under the direction of E. C. Rosenow. The results with the first of these were disappointing, but the second type gave marked benefit in a group of fifty-two children, 18 months to 12 years of age, studied over a four-year period. The attacks were said to be completely controlled in all but six.

In another four-year period a streptococcus vaccine derived from asthmatic patients was used both parenterally and orally in asthmatic children. With this product, sixty-eight of seventy-six patients were "almost completely controlled." The vaccine contained 2 billion organisms per cubic centimeter. The first dose was 0.3 c.c. subcutaneously. This was followed by 0.5 c.c. and 1 c.c. at intervals of one week. Thereafter 1 c.c. was given most cases at intervals of four weeks or upon the appearance of early symptoms of an attack. Mild local reactions occurred at times with the first few doses but "in most of the group no general reaction occurred." Local skin reactions with the vaccine by the scratch or patch methods were negative. Four illustrative cases are cited. Control studies are not mentioned.

The Practical Management of Eczema in Infants and Children. Osborne, E. D., Jordon, J. W., and Hallett, J. J.: New York State J. Med. **42**: 47, 1942.

A review of 124 cases of eczema in infants and children seen from 1938 to 1940 reemphasizes the views expressed by Osborne in an article published in 1938. Hypersensitivity to foods as a cause of infantile eczema is viewed as a minor factor which plays a part in not over 10 to 15 per cent of cases and is of considerably less importance in older children. Skin tests, either scratch or intradermal, are of exceedingly limited value. Patch tests are also of limited value

and are often incorrectly applied. They should be applied on an entirely normal skin area adjacent to an eczematous area in a manner which simulates clinical conditions as closely as possible. Better still is actual clinical trial such as wearing a glove or a fuzzy sweater or coat.

It is difficult to convince many parents of the importance of external contact and environmental allergens and this accounts for many failures. A thorough trial of four to eight weeks elimination of suspected substances is essential. The most common allergens of importance in eczema of infants and children are wool, silk, feathers, other epidermals, soap, laundry chemicals, tanning chemicals, dyes, lacquered objects, medications, fish oil, oils from fruit and vegetables, cosmetics, weeds, and plants. Local treatment is important and should generally be of a soothing nature, including the proper use of coal tar. Occasionally 50 to 75 rat units of x-ray in fractional doses is helpful in severe cases. In addition to a detective-like history and follow up, a visit to the home of the patient is indispensable.

Rhinology and Ophthalmology

UNDER THE DIRECTION OF FRENCH K. HANSEL, M.D., ST. LOUIS

Allergic Rhinitis. Analysis of One Hundred and Twenty-Eight Cases in Which the Rhinologist and the Allergist Collaborated. Woodward, F. D., and Swineford, O.: Arch. Otolaryng. **34**: 1123, 1941.

In view of the high incidence of allergy in chronic sinusitis, it is evident that the most satisfactory results are dependent upon adequate allergic management. A recent report on allergic rhinitis in which there was cooperation between the rhinologist and the allergist was presented by Woodward and Swineford. This paper illustrates the value of such cooperation. A group of 128 cases was studied. In 61 there were no significant complications. The remaining 67 showed the following: obstruction from the septum or turbinates and chronic ethmoiditis, 25; obstruction from nasal polyps, 16; pansinusitis and marked mucosal degeneration, 9; chronic maxillary sinusitis, 9; acute and subacute maxillary sinusitis, 4; and septal or turbinal obstruction without secondary infection, 4.

The opinion was expressed that, in general, a period of allergic management plus conservative rhinologic treatment should precede surgical intervention. In those few patients with long-standing severe asthma, bronchiectasis and physical and roentgenologic evidence of chronic sinus infection, they further state that little is to be gained by the postponement of surgical intervention. It was also emphasized that when a major surgical operation is necessary, it should be complete enough to effect a cure of the complications which necessitated it. In the group of 128 cases major surgical procedures were carried out as follows: obstruction and secondary ethmoiditis, 25; external operations, 18.

An evaluation of the results obtained in 89 cases which were followed up were as follows: 67 were still having nasal symptoms but 58 were from 75 to 100 per cent improved, 18 were 50 per cent improved, and 1 was reported less than 50 per cent improved.

Diagnosis of Allergy of the Nose and Paranasal Sinuses, With Particular Attention to the Atypical Types. Hansel, F. K.: Arch. Otolaryng. **34**: 1152, 1941.

In the diagnosis of allergy of the nose and paranasal sinuses with particular attention to the atypical types, Hansel calls attention to the importance of recog-

nizing those cases in which the symptoms are vague or fail to present the usual classical clinical picture. Particular attention is called to cases in which the symptoms consist mostly of stuffiness and postnasal discharge with little, if any, sneezing or anterior discharge. It is further emphasized that the diagnosis may depend to a great extent upon the demonstration of eosinophiles in the secretion. Because of the low-grade nature of these cases, the eosinophilia is not marked and is often difficult to demonstrate. A number of case reports are presented to illustrate the different types of atypical nasal allergy.

Some Dental Observations by an Otolaryngologist. Berry, Gordon: Ann. Otol., Rhin. and Laryng. **50**: 995, 1941.

Berry reports his more recent observations on the relation of dental infections to sinusitis. The author has published a number of very comprehensive reports on this subject and is therefore an authority on this important relationship. A number of dental roentgenograms and case reports illustrate the importance of relationship of antrum infections to dental pathology. He points out that even in those patients with nasal allergy, denal infection may be the cause of the occurrence of more than the usual number of acute colds or a complicating infection in the antrum.

Prophylaxis and Treatment of the Common Cold With Special Reference to Respiratory Vaccine. Veasey, C. A., Jr.: Ann. Otol., Rhin. and Laryng. **50**: 1168, 1941.

In the treatment of the common cold, Veasey recommends the use of small doses of respiratory vaccine administered by the intracutaneous method. He uses an intradermal injection of .016 c.c. of respiratory vaccine which has been diluted to one-third the original strength. He emphasizes the importance of individualizing the dosage according to the patient's sensitivity. He describes a syndrome which he designates as the bacterial allergic rhinitis. The chief complaints consist of nasal obstruction with thin, watery nasal secretion, with or without sneezing. The symptoms may be more or less continuous or paroxysmal with remissions. The patient appears to have a cold all the time, or frequently thinks he is getting a cold which never develops beyond a second watery secretion stage. Allergic survey fails to reveal a cause and eosinophiles are not demonstrated in the nasal secretion. Veasey states, however, that the secretion is so watery that the cells are difficult to demonstrate. In nasal allergies of this type with watery secretion it may be necessary to centrifuge the secretion or have the patient collect it in a bottle so that the mucoid masses may be collected for cytologic examination. It is evident that a very definite distinction must be made between infection and allergy and that the cytologic examination may be the only means of making the distinction.

Further Modifications of the Nasal Contact Test for Allergy. Vaughan, W. T., and Derbes, V. J.: Ann. Otol., Rhin. and Laryng. **50**: 1141, 1941.

Further modifications of the nasal contact test for allergy are presented by Vaughan and Derbes. This test requires the use of a mirror originally devised by Glatzel and a quantitative test by Lieb and Mulinos. The antigen is introduced into the nose by means of an inhalator similar to the Benzedrine type. The degree of nasal obstruction is measured by the length of time required for the condensation which appears on the cold mirror to evaporate.

Correspondence

The Editor,
THE JOURNAL OF ALLERGY.

In an article in the January, 1942, issue of The Journal of Allergy entitled "Allergens in Pollens" by Newell, the following appears on page 188: "Glycerol is irritating when injected into the skin so Moore and Unger recommended 5 per cent dextrose and 0.1 per cent cresol in distilled water for preparing pollen extracts. There is some objection to this solution in that molds are able to grow in it."

I wish to take exception to this statement. Since the extracting fluid was introduced in 1932, we have never had any infection nor have we ever noted any mold contamination.

Shortly after this solution was introduced, the formula was changed to the following:

U.S.P. dextrose	45 Gm.
Phenol	5 Gm.
U.S.P. sodium bicarbonate	2 Gm.
Distilled water q.s.	1,000 c.c.

The sodium bicarbonate was added to prevent the formation of some harmless glucosides which are apt to appear if the pollen solution is filtered too soon.

I might add that more and more men all over the country are using this solution because it does what was claimed for it originally; i.e., it is practically painless and it is as stable as any other extracting fluid, including glycerine, and more stable than such solutions as normal salt or Coca's solution.

I trust that you will print this letter in an early edition of the JOURNAL to correct what I believe is a gross error and misstatement of fact.

LEON UNGER.

Chicago, Jan. 30, 1942.